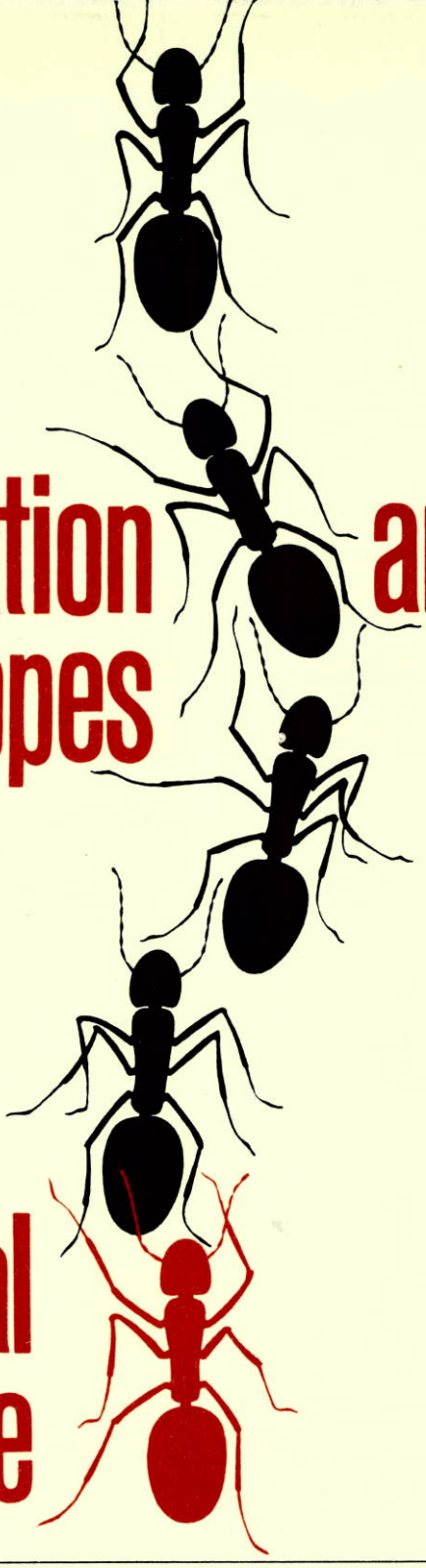
PROCEEDINGS OF A SYMPOSIUM,
ATHENS,
22-26 APRIL 1963
JOINTLY ORGANIZED
BY THE
IAEA AND FAO

Radiation and Radioisotopes Applied to Insects of Agricultural Importance



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1963

RADIATION AND RADIOISOTOPES APPLIED TO INSECTS OF AGRICULTURAL IMPORTANCE

PROCEEDINGS SERIES

RADIATION AND RADIOISOTOPES APPLIED TO INSECTS OF AGRICULTURAL IMPORTANCE

PROCEEDINGS OF THE SYMPOSIUM ON THE
USE AND APPLICATION OF RADIOISOTOPES AND RADIATION
IN THE CONTROL OF PLANT AND ANIMAL INSECT PESTS
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
AND HELD IN ATHENS, 22 - 26 APRIL 1963

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VIENNA, 1963

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FOREWORD

Since the pioneer work of the United States Department of Agriculture in the application of radiation and radioisotopes in the control of insect pests to cattle, many countries and organizations have pursued the advantages which might be gained in this field. Two years ago the IAEA organized the first international symposium in Bombay to study this problem, since when a considerable amount of basic research on the application of nuclear science in entomology and insect pest control has been undertaken. The potential gain of these studies, which would be in the form of an increased output of better food, is obvious to all Governments; hence the extensive international interest in the subject of this present Symposium, which was attended by 100 participants from 26 countries and 5 international organizations.

The proceedings consist of 37 papers presented by experts from 10 countries, together with a record of the discussions, and cover the use of radioisotopes in the study of the ecology of insects, such as their dispersal, migration and life-cycle. The application of radioisotopes to insecticides covers such subjects as labelling, application, uptake, translocation, metabolism, mode of action, and the determination of residues in plants and animals. The present position on the effects of radiation on insects is dealt with, including mutation, sterilization and the use of the sterile-male technique for the control and eradication of insect pests, and the need is emphasized for integration of chemical, biological, radiation and other methods of insect control.

The emphasis of this Symposium has been mainly on aspects of crop protection and it is hoped that the next symposium will also deal with aspects of livestock protection.

The sponsoring organizations wish to express their gratitude to the Government of Greece for its invitation to hold the Symposium in Athens, for its active assistance in the preparation and organization of the meeting, and for its generous hospitality to both participants and secretariat.

Appreciation is also due to Dr. E. Horber, of the Swiss Federal Experimental Station for Agriculture, Zürich-Oerlikon, who kindly assisted in the preparation of the Introduction to these proceedings and in the scientific editing of the discussion records.

EDITORIAL NOTE

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For the sake of speed of publication the present Proceedings have been printed by composition typing and photo-offset lithography. Within the limitations imposed by this method, every effort has been made to maintain a high editorial standard; in particular, the units and symbols employed are to the fullest practicable extent those standardized or recommended by the competent international scientific bodies.

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CONTENTS

INTRODUCTION 1

I. INSECT ECOLOGY: TRACER APPLICATIONS

Quelques emplois des radioéléments et des rayonnements en entomologie 5
G. Courtois et J. Lecomte

Tracer experiments on food exchange in ants and termites 25
K. Gösswald and W. Kloft

Use of radioactive tracers in the study of insect-plant relationships 43
D. A. Crossley, Jr.

Distribution of aerially applied Malathion-S^{35} in a forest ecosystem 55
R. H. Giles, Jr. and T. J. Peterle

Note préliminaire sur l'utilisation des radioisotopes dans l'étude des parasites du cotonnier en Afrique 85
R. Delattre

Use of isotopes for investigating the behaviour and ecology of insect pests in some recent studies 93
M. Sayeed Quraishi

Essai préliminaire avec ^{32}P sur la dispersion des adultes du Dacus oleae Gmel. 101
P. S. Orphanidis, C. D. Soultanopoulos et M. G. Karandeinos

Preliminary studies of the field movement of the olive fruit fly (Dacus oleae Gmel.) by labelling a natural population with radioactive phosphorus (P^{32}) 105
C. E. D. Pelekassis, P. A. Mourikos and D. N. Bantzios

Использование радиоизотопов и радиации в борьбе с насекомыми-вредителями растений и животных 115
С.В. Андреев, Б.К Мартенс, В. А. Молчанова, З.И. Самойлова

II. LABELLED INSECTICIDE STUDIES: TECHNIQUES

Some applications of radioisotopes to the study of the contamination of insects by insecticide solutions 135
C. T. Lewis

The application and measurement of labelled residual insecticides in some physico-chemical studies 147
F. T. Phillips

Radioautography in the study of radioisotopically tagged substances in insect control 155
D. L. Joftes

III. LABELLED INSECTICIDE STUDIES: TOXICOLOGY AND RESIDUES

Radioisotopes in the study of the fate of insecticides applied to animals and plants 171
F. W. Plapp, Jr. and D. A. Lindquist

Some problems in the determination of residues in plants and mammals 185
D. F. Heath

Évolution des dépôts superficiels, diffusion et dégradation de deux insecticides endothérapiques : le Demeton-S et l'Endothion, dans quelques plantes maraîchères 195
M. Hascoët

L'établissement des processus d'absorption et diffusion des insecticides systémiques au Populus x euroamericana Dode Guinier «Robusta» 211
I. Catrina, A. Popa, V. Constantinesco, O. Constantinesco, El. Constantinesco et C. Huluță
Radiotracer approaches to carbamate insecticide toxicology 223
J.E. Casida
Problems of application and action of Thiodan studied with S^{35}-labelled insecticide 241
K. Gösswald, E.-F. Schulze and W. Kloft
Применение радиоактивных изотопов в изучении процессов всасывания, распределения и выделения из животного организма некоторых инсектицидов 249
Г.В. Филатов, П.А Карташов, М.И. Мутин, И.А. Закамырдин, У.Я. Узаков
Studies on the selective toxicity of Schradan 255
Tetsui Saito

IV. INSECT METABOLISM: TRACER APPLICATIONS

Studies on the utilization, metabolism and function of sterols in the house-fly, Musca domestica 269
W.E. Robbins
Tyrosine metabolism in the blowfly, Calliphora erythrocephala 281
C.E. Sekeris
Étude de la pigmentation tégumentaire des insectes à l'aide de radioéléments 289
S. Fuzeau-Braesch

V. RADIATION STUDIES: PRINCIPLES AND APPLICATION OF THE STERILE-MALE TECHNIQUE

Effects of ionizing radiation on insects and other arthropods 301
William E. Stone
Eradication of white grub (Melolontha vulgaris F.) by the sterile-male technique 313
E. Horber
A technique of culturing the Olive Fly, Dacus oleae Gmel., on synthetic media under xenic conditions 333
K.S. Hagen, L. Santas and A. Tsecouras
Étude des populations et de dispersion de Ceratitis capitata Wied. (Dipt. Trypetidae) en Tunisie à l'aide des radioisotopes 357
F. Soria

VI. RADIATION STUDIES: SPECIFIC EFFECTS

Effects of radiation on germ cells of insects: dominant lethals, gametic inactivation and gonial-cell killing 367
R.C. von Borstel
The effects of gamma radiation on the ovaries of Dacus oleae Gmel. 387
Baccio Baccetti and Raffaella de Dominicis
Sterilization of Dacus oleae by gamma radiation 413
H. Thomou
Effects of variable dose-rates on radiation damage in the rust-red flour beetle, Tribolium castaneum Herbst 425
K.K. Nair and G. Subramanyam
Influence de l'irradiation sur les adultes de Sitophilus sasakii takahashi (Curculionidae) et leurs descendants 431
P. Laviolette et P. Nardon

Effects of gamma radiation on three species of Philippine insect pests ... 443
G. B. Viado and E. C. Manoto
Preliminary studies on irradiation of some common stored-grain insects in Pakistan 455
Heshamul Huque
Studies on the effects of gamma radiation on the different developmental stages of the Khapra beetle, Trogoderma granarium Everts. 465
K. K. Nair and G. W. Rahalkar
Radiosensitivity of various stages of Callosobruchus chinensis L. 479
M. Sayeed Quraishi and M. Metin
Disinfestation of dried figs by gamma radiation 485
C. P. Papadopoulou
Chairmen of Sessions and Secretariat of the Symposium 493
List of Participants 494
Author Index 501
Subject Index 503

INTRODUCTION

The contributions of the atomic sciences to entomological research are broad and continue to amaze and attract scientists interested in agricultural, silvicultural, industrial and medical entomology. Entomological research is an appropriate showcase to demonstrate the ever-widening range of the versatility of the atom's inherent possiblities.

A great deal of atomic energy research is dedicated to basic problems concerned not so much with immediate practical benefits, but with the development of techniques or materials which may be applied subsequently. Such exploratory studies have in many cases advanced to the stage of field tests. Break-throughs in one sector have to be considered against the increasing flow of background information in most other sectors which, through accretion, solidify the foundations for more striking progress in the future. This is the accepted procedure even for the more conventional and long-established branches of science.

From the papers presented and discussed at the Symposium at Athens it is evident, however, that the different fields of entomological research have shared unequally in the benefits of atomic energy.

Insect ecology depended on tracer application to study food exchange in social insects such as bees, wasps, ants and termites. Isotopes were also used to assess insect plant relationships quantitatively or to study the distribution of insecticides in a forest ecosystem. To trace the migration of aphids, grasshoppers, thrips, the Colorado potato beetle and soil insects, or the dispersal of the adult olive fly and the Mediterranean fruit fly, these insects were tagged with radioisotopes. The behaviour and biology of cotton insects, the Senn pest and malaria-carrying mosquitoes were observed with similar techniques. Yet in this field of application atomic energy research might profitably be intensified.

Isotopes have contributed to a high degree to the fast progress in insect physiology and insecticide research. The techniques of labelled insecticide and fungicide studies have been elaborated in order to study the contamination of insects by insecticide solutions or to calibrate dosage of minute amounts of labelled residual insecticides on test surfaces. Radioautography may be appreciated as a delicate tool of high resolution power to trace the radioisotopically tagged insecticides down to the cellular level if applied properly and evaluated conscientiously.

The increased emphasis given to insecticide and fungicide toxicology and residues is reflected in the widespread use of labelled compounds. They were used to follow the fate of insecticides and fungicides to determine the residues in plants and animals, to characterize surface deposits, areas of concentration and the diffusion and degradation of systemic insecticides in plants.

Insect metabolism, as well as insecticide toxicology, has felt the impact of isotope techniques, as shown by the key role played by radiotracers in studies on carbamate insecticides, other systemic and residual insecticides,

as well as on the metabolism and utilization of sterols in the house fly, tyrosine metabolism in the blowfly and tegumentary pigmentation in Orthoptera.

Irradiation of insects has received wide attention in all aspects, from the fundamentals of genetics, through the different approaches of exploratory development to technology and successful application to limited areas or to country-wide insect-pest eradication programmes. Radiation studies on specific effects have been carried out on male and female germ cells in Diptera, Hymenoptera, Coleoptera and Hemiptera in order to measure variations in response and sensitivity to radiation at different stages of oogenesis and spermatogenesis. The principles and applications of the irradiated-male technique were demonstrated on a wide variety of insects and other arthropods. Among the Diptera studied are a horn fly, the Mediterranean fruit fly, the melon fly, the Mexican fruit fly, the olive fly, the oriental fruit fly, several species of mosquito, the screw-worm and the stable fly. In Coleoptera, data are now available on the boll weevil, the Colorado potato beetle, the cowpea weevil, the grain-borer, the Mexican bean beetle, the rice weevil, the rust-red flour beetle, the tomato lady beetle and a white grub. Among the Lepidoptera were considered the European corn-borer, the gypsy moth, the pink bollworm and among other arthropods the Lone Star tick and a scorpion. Great efforts are directed towards the control of the olive fly. Mass-rearing techniques need to be elaborated before it will be possible to release sufficient numbers of irradiated males.

Insect pests of stored commodities are a very important problem, especially in sub-tropical and tropical countries; this was reflected in the number of contributions in that area of research. Putting into practice the irradiation of foodstuffs, e.g. the irradiation of grain to render it insect-free, depends not only on acquiring the necessary basic radiobiological knowledge and on advances in irradiation technology, but also on health and safety considerations.

The harrowing question of whether arthropods are able to build up resistance to radiation as they do to insecticides remains to be answered.

An attempt has been made by the sponsoring organizations to assemble representative experts at regular intervals to review progress in the field. The papers selected for the Athens Symposium varied greatly in scope, approach and level of contribution, but this too is representative of the stages reached in the various aspects of such research.

It has been our aim to reduce the time-lag between the meeting and the publishing of the Proceedings to a minimum. Only then can the Proceedings be a valid tool of communication enhancing or catalysing progress instead of being relegated to a cemetery for forgotten facts or obsolete ideas. The Symposium reported here should be regarded as a milestone marking a successful research period and staking out the directions in which more effort has to be concentrated before measurable progress can be expected. It can then be assumed that the Athens Symposium of 1963 is serving its purpose as fully as the previous one held in Bombay in 1960*.

* Proceedings published as Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 307 pp.

I.

INSECT ECOLOGY: TRACER APPLICATIONS

QUELQUES EMPLOIS DES RADIOÉLÉMENTS ET DES RAYONNEMENTS EN ENTOMOLOGIE

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Abstract — Résumé — Аннотация — Resumen

SOME USES OF RADIOISOTOPES AND RADIATIONS IN ENTOMOLOGY. The paper reviews the applications of radioisotopes in entomology that have been developed at our two centres during the last few years.

Early work (Au^{198}-labelling) related to the bee and more particularly to the radius of dispersion of worker bees from a colony. After investigations on the individual dose received in tagging of this kind, the radioresistance of the bee was determined, the lethal dose being estimated at about 90 kr. Au^{198} was also used to study exchange of food within a bee-hive. On the other hand, P^{32} was used for studies of exchange of food, in small hives, between individuals of different functions (males, workers and queens) or different colonies. Similar trophallaxic studies have recently been performed on wasps.

Au^{198} was likewise the basic radioisotope used in work on ant's nests. The most interesting finding from one of the early studies was that exchange of food takes place between nests more than 50 m apart and belonging to different species (Formica rufa and Formica polyctena). A later study, in which an ant run and not the nest itself was labelled, revealed a division of responsibility within the nest: the tagged ants were found invariably to explore the same run and to have little contact with other individuals of the same colony. In the same experiment abnormal radioactivity was noted in the ants before labelling, due in particular to $(Sr+Nb)^{95}$. This discovery would seem to point to accumulation of radioactive fall-out in ant's nests. At a period of low fall-out, natural radioactivity attributed to K^{40} was observed and was used for purposes of potassium determination in ants and bees.

An attempt was made to label acridians with Ir^{192} and the findings are described in the paper.

Lastly, an autoradiographic study has been made of the distribution of certain radioisotopes (P^{32} and S^{35}) in the body of the bee.

QUELQUES EMPLOIS DES RADIOÉLÉMENTS ET DES RAYONNEMENTS EN ENTOMOLOGIE. Le mémoire passe en revue les applications des radioéléments en entomologie qui ont été mises au point au CEN et à l'INRA durant ces dernières années.

Les premiers travaux concernèrent l'abeille, plus particulièrement la dispersion des butineuses en provenance d'une colonie; l'étude a été réalisée par des marquages à ^{198}Au. Suite à ces considérations sur la dose reçue par l'individu dans de tels marquages, la radiorésistance de l'abeille a été déterminée et la dose létale estimée à 90 kr environ. ^{198}Au a également servi à étudier les échanges de nourriture à l'intérieur d'une ruche. Par contre, c'est ^{32}P qui fut utilisé pour des études d'échanges de nourriture à l'intérieur de ruchettes entre individus de fonctions (mâles, ouvrières, reines) ou de colonies différentes. Des études analogues de trophallaxis ont récemment été faites sur des guêpes.

^{198}Au a été également le radioélément de base de travaux sur les fourmilières. Le résultat le plus intéressant d'une première étude a été la découverte d'échanges de nourriture entre fourmilières distantes de plus de 50 m et d'espèces différentes (Formica rufa et Formica polyctena). Dans une deuxième étude, par marquage d'un chemin de fourmis et non de la fourmilière elle-même, on a mis en évidence une division des responsabilités à l'intérieur de la fourmilière, les fourmis marquées prospectant toujours le même chemin et n'ayant que peu d'échanges avec les autres individus de la même colonie. Dans cette même expérience, on a constaté avant tout marquage une radioactivité anormale des fourmis, due notamment à $^{95}(Sr + Nb)$. Cette découverte aurait tendance à montrer un amassage des retombées radioactives dans les fourmilières. En période

de faibles retombées, une radioactivité naturelle attribuée au ^{40}K avait été constatée et avait servi à faire un dosage du potassium dans les fourmis et dans les abeilles.

Un essai de marquage d'acridiens à ^{192}Ir a été fait et les résultats obtenus sont décrits dans le mémoire.

Enfin, une étude de la répartition de certains radioisotopes (^{32}P, ^{35}S) dans le corps de l'abeille au moyen de la méthode autoradiographique a été effectuée.

НЕКОТОРЫЕ ВИДЫ ПРИМЕНЕНИЯ РАДИОЭЛЕМЕНТОВ И ОБЛУЧЕНИЯ В ЭНТОМОЛОГИИ. Статья содержит обзор методов применения радиоэлементов в энтомологии, разработанных в наших двух центрах в течение последних лет.

Первые работы относились к пчелам и в особенности к дальности полетов пчел-сборщиц из пчелосемьи. Исследования проводились с помощью мечения изотопом Au^{198}. Продолжением работ по определению дозы, полученной особью в процессе мечения, явилось определение радиорезистентности пчелы и летальной дозы, лежащей в пределах 90 000 р. Au^{198} был также использован для исследования обмена пищей внутри улья. Вместе с тем, для изучения обмена пищей внутри ульев между рабочими особями (мужскими особями, рабочими пчелами, матками) или между различными роями применялся P^{32}. Недавно аналогичные исследования трофилаксии были проведены на осах.

Au^{198} был также основным радиоэлементом в работе с муравейниками. Наиболее интересным результатом первого исследования явилось открытие обмена пищей между муравейниками, отстоящими друг от друга на расстоянии свыше 50 м, и разными видами муравьев (Formica rufa и Formica polyctena). В процессе второго исследования, с помощью мечения пути движения муравьев, в не самого муравейника, было выявлено распределение обязанностей внутри муравейника. Меченые муравьи следовали по одному и тому же маршруту и редко обменивались с другими особями той же колонии. В процессе этого опыта уже до мечения была установлена аномальная радиоактивность муравьев, обусловленная прежде всего наличием $(Sr + Nb)^{95}$. Это открытие говорит о тенденции к накоплению в муравейниках радиоактивных веществ из осадков. В период слабых осадков определяли естественную радиоактивность, связанную с наличием K^{40}, и ее использовали для определения содержания калия в организме муравьев и пчел.

Была предпринята попытка мечения саранчовых насекомых. Приводятся полученные результаты.

Наконец, было проведено исследование распределения некоторых радиоизотопов (P^{32} и S^{35}) в организме пчел с помощью метода авторадиографии.

ALGUNAS APLICACIONES DE LOS RADIOELEMENTOS Y DE LAS RADIACIONES EN ENTOMOLOGÍA. La memoria reseña las aplicaciones de los radioelementos en entomología, ensayadas en los últimos años en los dos centros a que pertenecen los autores.

Los primeros trabajos se dedicaron a la abeja, estudiándose particularmente con marcaciones de ^{198}Au la dispersión de las colectoras provenientes de un enjambre. Tras considerar la dosis recibida por cada individuo como resultado de las marcaciones, se determinó la radiorresistencia de la abeja y se calculó que la dosis letal ascendía a unos 90 kr. También se utilizó el ^{198}Au para estudiar el intercambio de alimentos en el interior de una colmena. Ahora bien, para estudiar el intercambio de alimentos dentro de pequeñas colmenas entre individuos de función (machos, obreras, reinas) o enjambres diferentes se utilizó el ^{32}P. Recientemente se han hecho estudios análogos de trofalaxia sobre las avispas.

El ^{198}Au se ha empleado también como radioelemento básico para el estudio de hormigueros. El resultado más interesante del primer estudio efectuado fue el descubrimiento de un intercambio de alimentos entre hormigueros distantes más de 50 m y correspondientes a especies diferentes (Formica rufa y Formica polyctena). En otro estudio, se marcó un camino de hormigas en lugar del hormiguero y se pudo comprobar que en el interior de éste existe una división de funciones, pues las hormigas marcadas exploraban siempre el mismo camino y tenían muy escasos contactos con los demás individuos de la colonia. En este mismo experimento, antes de proceder a la marcación se observó en las hormigas una radiactividad anormal, debida en particular al $^{95}(Sr + Nb)$. Esto parece demostrar que las precipitaciones radiactivas se acumulan en los hormigueros. En período de precipitaciones escasas se había podido observar una radiactividad natural imputable al ^{40}K, que sirvió para dosificar el potasio en las hormigas y en las abejas.

Los autores han ensayado la marcación de acrídidos con ^{192}Ir y describen en la memoria los resultados obtenidos.

Se ha hecho también un estudio de la distribución de ciertos radioisótopos (^{32}P y ^{35}S) en el cuerpo de la abeja por medio del método autorradiográfico.

Le présent article passe en revue les applications des radioéléments dans l'écologie et la biologie des insectes, développées dans nos deux centres ou avec leur collaboration, durant ces dernières années.

I. ÉTUDE SUR LES ABEILLES

1. Etude du secteur de butinage [1, 2, 3]

La première étude réalisée a été celle du rayon d'action et de la dispersion des abeilles butineuses en provenance d'une ruche. Cette étude, commencée dès 1958, a été effectuée grâce à un marquage total de l'ensemble de la population de la colonie, soit environ 40 000 individus. Différentes considérations de caractéristiques radioactives, de nocivité et de facilité d'assimilation nous ont fait choisir comme traceur, incorporé à la nourriture, le ^{198}Au (période: 2,7 j), émetteur entre autre d'un gamma de 0,411 MeV.

L'activité d'environ 1 μc par individu a été choisie en fonction de la période biologique de ^{198}Au (2 à 3 j) et des hétérogénéités de marquage d'une abeille à l'autre. Ainsi une quarantaine de millicuries de ^{198}Au, permet-il de faire le marquage d'une colonie entière dont chaque individu peut être repéré pendant 4 à 5 j après début du marquage. Les échanges de nourriture entre insectes aidant, au bout d'un temps variable, mais toujours inférieur à 48 h, la quasi totalité des abeilles possède une activité suffisante pour permettre la détection dans la nature. Dans le secteur de butinage, la détection s'effectue à l'aide d'un détecteur portatif à scintillation (cristal NaI(Tl) 1''1/2 × 1''), chaque insecte étant préalablement attrapé à l'aide d'un filet à papillon à mailles fines. Ce procédé permet l'examen d'insectes posés dans des endroits inaccessibles à la sonde détectrice, branches hautes des arbres par exemple, et donne loisir d'examiner attentivement les cas douteux.

Le terrain expérimental toujours le même comportait un rucher de trente colonies parmi lesquelles était choisie celle marquée, huit autres ruches se trouvant dans un rayon d'environ 1 km.

En 1958, 17 butineuses marquées furent retrouvées sur 1027 examinées; en 1960, le pourcentage fut de 81 butineuses marquées sur 2442 ouvrières attrapées. On constate que dans les conditions de l'expérience, aucune butineuse ne dépasse sensiblement 1 km de distance, le gros de la troupe se trouvant à l'intérieur d'un cercle de 600 m de diamètre (figure 1 et tableau I). D'autre part, l'hétérogénéité de la distribution dans l'espace des butineuses marquées est très frappante. Ainsi, nous avons pu trouver un champ de crépis où la proportion d'abeilles marquées était de 33%. Un certain nombre de facteurs conditionnant le choix des secteurs de butinage a pu ainsi être mis en évidence tel que l'effet répulsif du relief et celui des grandes étendues stériles.

En 1959, un marquage similaire fut tenté, mais avec des conditions météorologiques mauvaises, abondantes chutes de pluie et température assez basse. Malgré une forte miellée de robiniers et bien que le sirop de sucre ait été absorbé plus rapidement que dans les autres cas, les pourcentages de butineuses marquées furent loin d'être aussi importants et ne

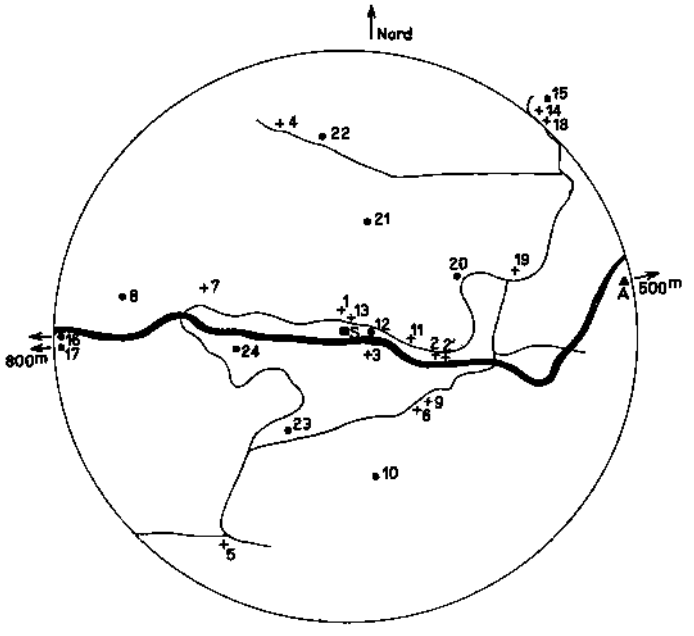

Figure 1

Etude du secteur de butinage. *

- Rivière
- Route
- + Abeilles marquées
- ● Pas d'abeilles marquées
- ▲ Fleurs sans abeilles
- ■ Emplacement de la ruche
- S Source
- ϕ Diamètre 1000 m

dépassèrent pas 70%, l'activité individuelle étant d'ailleurs plus faible. Une seule abeille active put être retrouvée sur le secteur de butinage.

2. Résistance au rayonnement gamma de l'abeille ouvrière [4]

Lors de tels marquages, nous nous sommes inquiétés de connaître les doses reçues par abeille et l'influence que pourraient avoir de telles doses sur le comportement des individus. Un calcul approximatif, considérant que l'émission β de 1 MeV de ^{198}Au est dissipé dans un individu, montre que la dose maximum reçue par une abeille marquée par 1 μc de ^{198}Au est de 600 r.

Nous avons donc soumis des ouvrières à l'action croissante du rayonnement du ^{60}Co (450 c de ^{60}Co fournissant une dose d'environ 100 000r/h sur un volume de 500 cm^3).

Les abeilles ainsi irradiées furent comparées à deux cagettes de témoins. Aucun effet décelable n'apparaît en-dessous de 18 000 r, au moins

* Les chiffres renvoient au tableau I.

TABLEAU I *

ÉTUDE DU SECTEUR DE BUTINAGE

Prélèvements	Plantes visitées	Abeilles capturées	Abeilles marquées
1	Ciste (Cistus albidus)	125	5
2	Crepis sp.	100	33
2'	Mélilot (Melilotus officinalis)	100	8
3	Sainfoin (Onobrychis sativa)	212	3
	Ciste - Thyn (Thymus sp.)		
4	Moutarde (Synapis arvensis)	101	1
5	Renoncule (Ranunculus sp.)	121	1
6	Acacia	150	1
7	Ciste	108	7
8	Renoncule, Vesce, Moutarde	3	0
9	Ciste	100	10
10	Cistes (C. albidus et Monspelliencis)	100	0
11	Acacia	103	6
12	Acacia	75	0
13	Thym	56	2
14	Thym	57	1
15	Vesce (Vicia sativa)	122	0
16	Sainfoin	105	0
17	Thym	105	0
18	Ciste	125	2
19	Ciste	49	3
20	Ciste	40	0
21	Ciste	101	0
22	Crepis, Mélilot, Ciste	100	0
23	Thym	29	0
24	Sainfoin	111	0

* Voir figure 1.

au cours des huit premiers jours, soit à des doses bien supérieures à celles reçues lors d'expériences par traceurs radioactifs. Entre 18 000 et 70 000 r, les dommages subis semblent dépendre des conditions physiologiques des individus, les abeilles les plus âgées semblant plus sensibles au rayonnement.

La dose létale (mort de la moitié des individus) semble être atteinte vers 90 000 r, 200 000 r étant la première dose qui entraîne la mort immédiate de tous les insectes.

3. Echange de nourriture à l'intérieur d'une ruche [5, 6]

A l'intérieur d'une ruche expérimentale d'observation formée d'un cadre unique et ayant des parois transparentes, une certaine quantité de ^{198}Au a été fournie à cinq à huit butineuses. La paroi de cette ruche étant munie d'un quadrillage de repère, une prospection a été continuellement faite après le nourrissement. L'examen a été fait à l'aide d'un détecteur à scintillation, sans collimateur, l'expérience ayant montré que la mesure était peu affectée par le rayonnement des zones voisines.

Contrairement à ce qu'on aurait pu attendre, les échanges de nourriture ne se font pas à partir du nourrisseur vers le centre de la grappe d'abeilles, mais il apparaît de manière certaine que les ouvrières, après avoir rempli leur jabot de miel radioactif, se dirigent rapidement vers le centre de la grappe, point privilégié à partir duquel s'effectue par la suite la presque totalité des échanges et la diffusion de la nourriture à travers l'ensemble de la population. Ce point correspond au centre thermique de la ruche où pendant la période d'hivernage se tient généralement la reine. Il fut également constaté que, contrairement à ce qu'on obtenait en période active en été, le nombre d'individus ayant ingurgité de la nourriture active était faible, soit 15 à 20% des individus. Il est vrai que la radioactivité avait été initialement fournie à un nombre très restreint d'insectes.

Sur ces bases, Mlle SALLERON [6] a étudié les échanges de nourriture entre individus de castes (reines, butineuses, mâles, etc.) ou de colonies différentes. Le travail a été effectué en ruchettes expérimentales, adossées l'une à l'autre et munies de trous permettant l'échange de nourriture sans autoriser le passage d'un insecte d'une ruchette à l'autre. Dans ce cas, les abeilles n'ayant pas accès à l'extérieur, le traceur employé fut le ^{32}P, émetteur β pur, sous forme de phosphate monosodique. Il fut incorporé à une nourriture solide à base de miel et de sucre préférable pour l'entretien des abeilles élevées en cagettes. Les comptages furent effectués soit sur des insectes morts soit sur des insectes vivants à l'aide d'un compteur G-M cloche de 1,5 mg/cm^2 de paroi. Trois questions furent étudiées à l'aide de cette technique:

1° Echange entre ouvrières

De par les études faites, il n'est pas apparu évident, contrairement à l'opinion déjà émise, que des ouvrières préfèrent échanger de la nourriture avec des ouvrières de la même colonie plutôt qu'avec des étrangères en provenance de ruche différente. Si cette tendance existe, elle est faible. Il semble que les échanges s'effectuent plus volontiers avec les abeilles

sœurs pendant les deux premiers jours, pour s'inverser ensuite et se faire plutôt avec les étrangères. Peut-être les nourrices moins généreuses avec elles au début les ont-elles obligées à demander davantage?

2° Echange entre mâles et ouvrières

Ce travail a montré sans ambiguïté que les mâles sont capables dans une certaine mesure de se nourrir seuls mais qu'en présence d'ouvrières, ils préfèrent se faire nourrir par ces dernières. D'autre part, il y a transfert de radioactivité des mâles vers les ouvrières, ce transfert pouvant être dû dans certains cas à l'absorption par les ouvrières des régurgitations des mâles déposés sur le substrat, dans d'autres cas à un échange direct de nourriture, échange qui a d'ailleurs pu être directement observé.

3° Echange entre reines et ouvrières

L'existence d'un transfert de radioactivité des reines aux ouvrières paraît maintenant une chose certaine. D'autre part, ces échanges ne sont le fait que d'un très petit nombre d'abeilles spécialisées même au sein de colonies expérimentales restreintes composées d'une vingtaine d'ouvrières.

4. Répartitions de certains radioéléments dans le corps de l'abeille.

Par la méthode autoradiographique, on s'est proposé de localiser les radioéléments dans le corps de l'abeille. Un certain temps après ingurgitation du traceur, les abeilles sont placées à −35° et incluses dans une solution de gelose. L'insecte est ensuite coupé en totalité au moyen d'une scie circulaire très fine; sur la coupe ainsi obtenue on place le film dont l'exposition a lieu à −35°.

Cette technique a été utilisée pour le ^{32}P sous forme de phosphate monosodique et pour le ^{35}S sous forme de $^{35}SO_4H_2$, introduits tous deux dans de la nourriture solide.

Le rayonnement trop énergique du ^{32}P ne permit pas l'obtention de bons clichés comme par exemple celui de la figure 2 qui montre la localisation du ^{35}S.

II. ÉTUDE SUR LES FOURMIS

Les résultats obtenus à l'aide des radioéléments dans l'étude des abeilles nous ont incités à l'utiliser pour d'autres insectes sociaux et notamment pour les fourmis.

1. Transmission d'isotopes radioactifs entre deux fourmilières d'espèces différentes [7] .

Chauvin étudiant depuis six ans de nombreuses fourmilières d'un certain bois, le terrain nous apparu propice à l'étude de la transmission de nourriture entre fourmilière à l'aide de traceurs radioactifs.

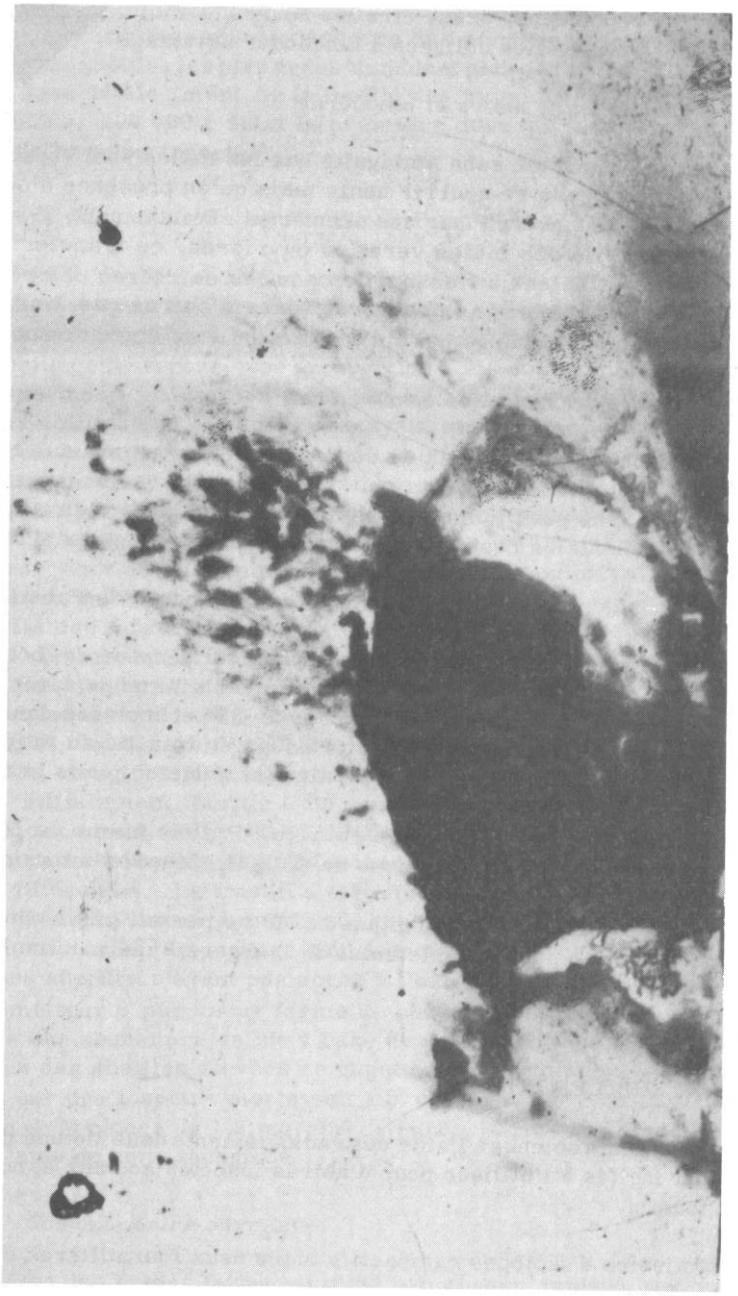

Figure 2

Autoradiographie d'une abeille après absorption de ^{35}S.

Une grosse fourmilière de Formica polyctena fut marquée, un soir, en versant sur elle 50 mc de ^{198}Au mélangées à 50 cm^3 d'une solution sucrée épaisse. Si l'on considère cette colonie comme peuplée d'un million d'individus et si l'activité initiale se répartit entre toutes les fourmis, nous arrivons à une activité de 0,05 μc par insecte très largement décelable même par le moins sensible des détecteurs dont nous disposions: un détecteur portatif à scintillation et un ensemble de comptage à scintillation à poste fixe, muni d'un cristal creux 1"3/4 × 2".

Le lendemain matin, le comptage de prélèvements de fourmis sur les différentes pistes partant de la fourmilière marquée donnent des résultats variables, l'activité des individus du milieu des pistes étant toujours plus grande que celle des extrémités. Ces inégalités s'atténuent d'ailleurs avec le temps mais restent cependant notables. On constate sur une fourmi particulièrement radioactive que l'activité est interne, l'abdomen portant 99,9% de l'activité totale.

Le résultat le plus intéressant obtenu lors de ces essais a été la découverte d'une certaine activité dans trois fourmilières d'une espèce voisine, Formica rufa, situées à une ciquantaine de mètres du nid des polyctena. Nous avons pu exclure l'hypothèse que les rufa se contaminaient en traversant les pistes de polyctena car ces pistes elles-mêmes sont inactives, ensuite la population de polyctena qui s'y déplace est si dense qu'on ne voit pas la possibilité pour une fourmi d'une autre espèce de la traverser sans se faire houspiller d'importance. Reste alors des attaques possibles des polyctena par les rufa qui pourraient les dévorer et emporteraient ainsi dans leurs colonies des matières radioactives. Mais nous n'avons pu constater ces attaques dans la nature et d'autre part, les auteurs généralement s'accordent à admettre que c'est polyctena, l'espèce la plus agressive, qui attaquerait rufa. Peut-être aussi les rufa pourraient-elles emporter des matériaux de construction souillés des excréments radioactifs de polyctena; nous sommes dans l'incapacité d'infirmer cette dernière hypothèse que nous considérons pourtant comme assez improbable. On pourrait penser aussi que les rufa fréquenteraient les mêmes pins que polyctena et lècheraient les mêmes pucerons pour en obtenir du miellat, mais nous n'avons pu, jusqu'ici, mettre en évidence des pistes de rufa se dirigeant vers les pins de polyctena et nous n'avons jamais vu de rufa sur le tronc de ces pins; nous pensons donc qu'il faut rejeter l'hypothèse d'une régurgitation de matières radioactives auprès des pucerons qui seraient ensuite absorbés par les ouvrières de rufa. Il semble donc assez probable qu'il y ait échange de nourriture entre des fourmis d'espèce différente appartenant à des fourmilières voisines.

2. Echanges de nourritures entre individus de la même fourmilière [8]

Durant l'été dernier, un nouveau marquage a été réalisé en plaçant la nourriture non plus sur la foumilière, mais au milieu d'une piste de butinage à environ 50 m du nid.

Les comptages effectués sur des prélèvements de 50 fourmis ont toujours montré une très nette supériorité du taux de comptage pour les prélèvements effectués sur la piste initialement marquée (voir fig. 3) et les

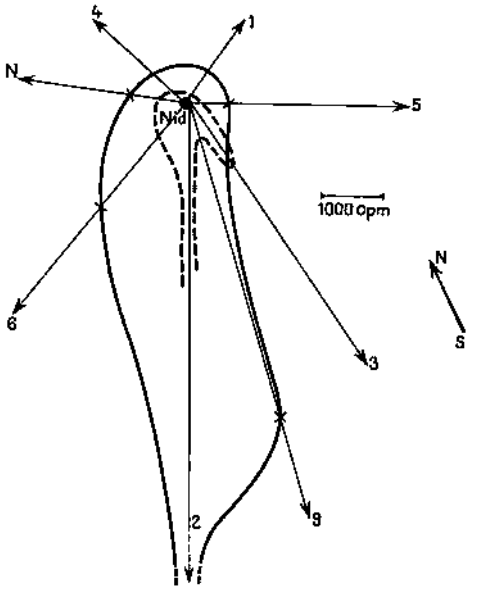

Figure 3

Représentation schématique des pistes rayonnant autour du nid.
Sur chaque piste, on trace un vecteur proportionnel à la moyenne des cpm pour 50 fourmis. Les deux tracés obtenus en réunissant l'extrémité distale de ces vecteurs correspondent à la distribution quantitative de la radioactivité pour deux journées de mesure. Le marquage a été effectué sur la piste 2. Le vecteur de la piste 2 devrait, pour la journée du 25, se prolonger de 92,5 et, pour la journée du 26, de 130 fois la valeur du segment symbolisant 1000 cpm
- - - Journée du 25
—— Journée du 26

échanges de nourriture restent très faibles entre ouvrières travaillant sur des pistes différentes. On observe cependant une augmentation générale des taux de comptage d'un jour à l'autre et la transmission de nourriture est lente, mais certaine, et intéresse l'ensemble de la population de la fourmilière.

Il semble donc possible de supposer l'existence à l'intérieur de la fourmilière de groupes de travail ayant relativement peu de contact pendant les périodes de butinages. Le fait que cette coupure soit moins forte entre les chemins voisins peut tenir au fait que les groupes de travail butinant dans des secteurs voisins occupent dans la fourmilière un emplacement généralement voisin, soit être due à ce qu'un certain nombre de butineuses peuvent changer de lieu de travail en le faisant toutefois dans des limites relativement étroites.

3. Teneur en potassium des fourmis et des abeilles

Lors des expériences précédemment décrites, nous avions constaté que le bruit de fond relevé au scintillomètre portatif sur les fourmilières était légèrement supérieur de l'ordre de 1 choc/s au bruit de fond ambiant.

Des prélèvements effectués ont été analysés et comptés au détecteur à bas bruit de fond (ensemble Philips à couronne d'anticoïncidence, détecteur G-M à circulation de bruit de fond 0,8 cpm environ).

La radioactivité trouvée a été attribué au ^{40}K. Elle correspondrait à une teneur d'environ 1% en poids de potassium dans la fourmi.

Une étude analogue chez l'abeille conduit à une teneur de 1,5% en poids dans l'abeille préalablement desséchée.

4. Les fourmis indicateurs possibles de retombées radioactives [9].

Cependant lors d'une dernière expérience, fin juillet 1962, nous constatons avant tout marquage une différence de bruit de fond entre fourmilière et ambiance de 25 chocs/s très visible avec le détecteur portatif et ceci sur toutes les fourmilières sans exception. La radioactivité se trouve dans les fourmis et non dans les matériaux constitutifs de la colonie, car une fourmilière désertée depuis un mois est la seule à ne pas présenter ce phénomène.

Un prélèvement de 880 g de fourmis est suivi d'un examen à l'aide d'un spectromètre gamma 60 canaux. Le spectre obtenu (fig. 4) présente trois pics:

- un pic à 780 keV ± 20 keV attribuable à la filiation $^{95}(Zr+Nb)$ (période: 65 j);
- un pic aux alentours de 140 keV attribuable au ^{141}Ce (période: 33 j);
- un léger pic vers 500 keV attribuable au ^{103}Ru (période: 40 j).

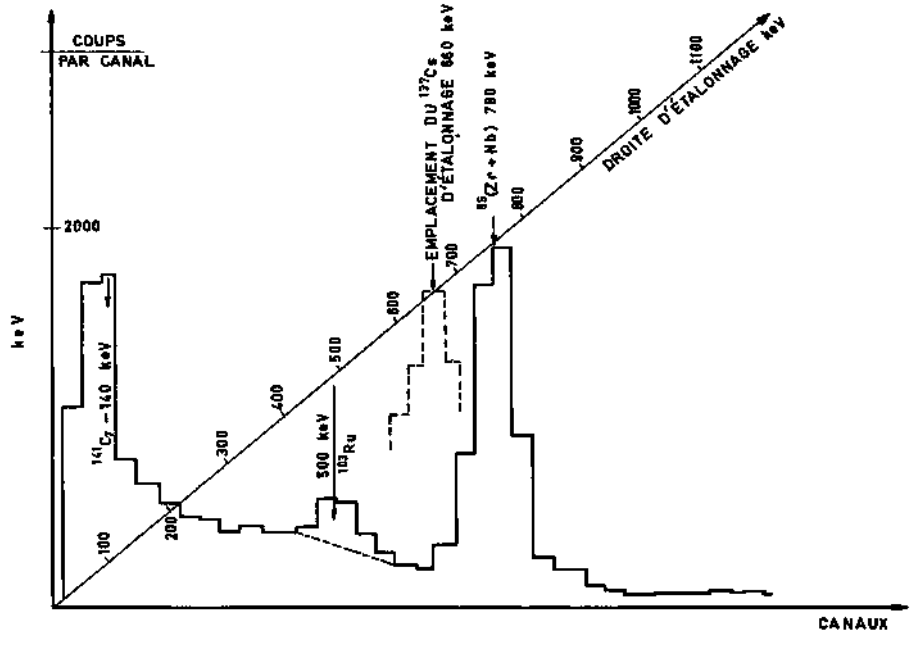

Figure 4

Spectre d'un prélèvement de fourmis en juillet 1962.
880 g de fourmis. Stockage 15 min. Bruit de fond déduit.

Une évaluation de l'activité due au $^{95}(Zr + Nb)$ donnait $3,5 \cdot 10^{-9}$ c/kg de fourmis.

Le mécanisme par lequel les fourmis fixent les retombées paraît en liaison directe avec leur habitude de prélever les excréments sucrés des pucerons des arbres, ou miellat. Les multiples gouttelettes gluantes ainsi exposées à l'air doivent capter une très grande quantité de poussières atmosphériques et, par conséquent, celle des retombées. D'ailleurs, les gouttelettes de miellat qui tombent sur les feuilles sont récoltées aussi par les abeilles, et l'on sait depuis longtemps que le miellat d'abeilles est bien plus riche en poussières que les miels floraux (où les nectaires sont bien abrités en général, ou même au fond de cavités étroites). Il est possible aussi et même probable que la résine des conifères fixe aussi ces poussières, et que les fourmis en entraînent quelque peu au cours de leurs innombrables marches et contremarches, sur les troncs. D'autre part, le fait que les fourmilières abandonnées ne montrent pas d'activité supérieure au bruit de fond, même quand l'abandon est très récent (c'était le cas dans notre premier prélèvement) prouve que les fourmis ne défèquent pas à l'intérieur de la fourmilière: on le pensait depuis longtemps, mais nous croyons que, pour la première fois, la démonstration en est apportée dans la nature.

Quant à l'importance des fourmis comme détecteurs des retombées, elle reste à évaluer, par rapport aux méthodes traditionnelles de comptage du bruit de fond sur échantillons de végétaux prélevés au hasard. Nous pensons qu'il serait nécessaire pour évaluer la sensibilité de la méthode par les fourmis, de suivre comparativement l'histoire d'une retombée dans la fourmilière, par comparaison avec les méthodes classiques.

III. ÉTUDES SUR LES ACRIDIENS MIGRATEURS*

A la suite d'un problème posé par M. Descamps du Centre de défense des cultures, nous avons regardé les possibilités de marquage radioactif d'un grand nombre d'acridiens (jusqu'à 500 000) pendant une période suffisamment longue de deux à trois mois pour faire une étude d'un secteur de grégarisation au Niger.

Le problème posé conduisait à prendre un radioélément de période radioactive et biologique suffisante. Les essais en laboratoire ont été effectués avec de ^{192}Ir sous forme d'une solution de chloroiridate d'ammonium dispersée sur de jeunes feuilles de blé servant de nourriture aux lots d'insectes étudiés. Chaque lot était constitué de 100 insectes d'âge différent d'un lot à l'autre (fig. 5).

Les résultats obtenus peuvent être divisés en éléments favorables et défavorables à un marquage de grande envergure.

1. Eléments favorables

Période apparente satisfaisante: La période apparente est de l'ordre de six jours indépendamment de l'âge des insectes; cependant la dispersion

* Etudes effectuées en collaboration avec M. Descamps, Centre de défense de cultures.

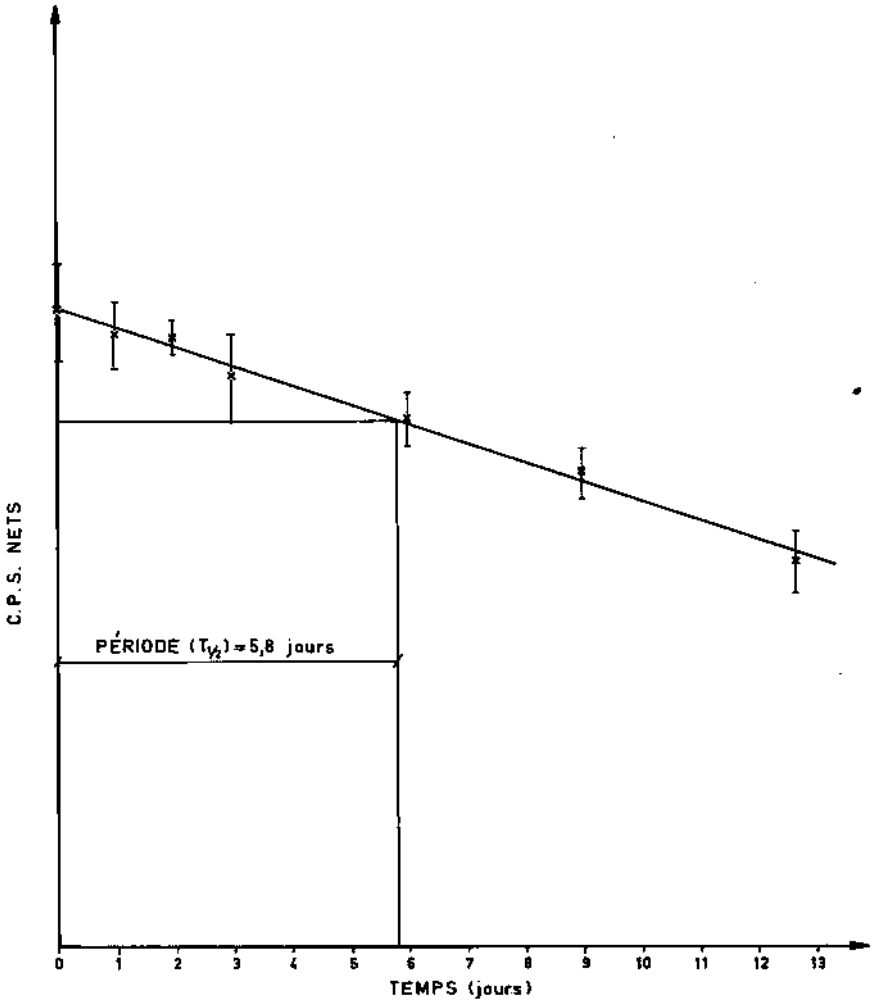

Figure 5

Période biologique de ^{192}Ir chez la sauterelle.

des résultats semble plus élevée pour les insectes les plus jeunes. D'autre part, suivant les experts, le métabolisme étant moins important dans la nature, il se pourrait que dans un marquage dans la nature, la période apparente soit encore plus importante (fig. 6).

Comportement satisfaisant des acridiens marqués: Il n'y eut pendant la période étudiée, ni mortalité ni changement de comportement notable.

Dispersion raisonnable de l'activité absorbée: Il y eut peu de sauterelles non marquées et aucune ayant absorbé 10 fois plus que la moyenne.

Individus non contaminants en dehors des excréments: Plus de la moitié de l'activité reste localisée dans la tête.

Pas de diminution de l'activité au passage à l'état adulte.

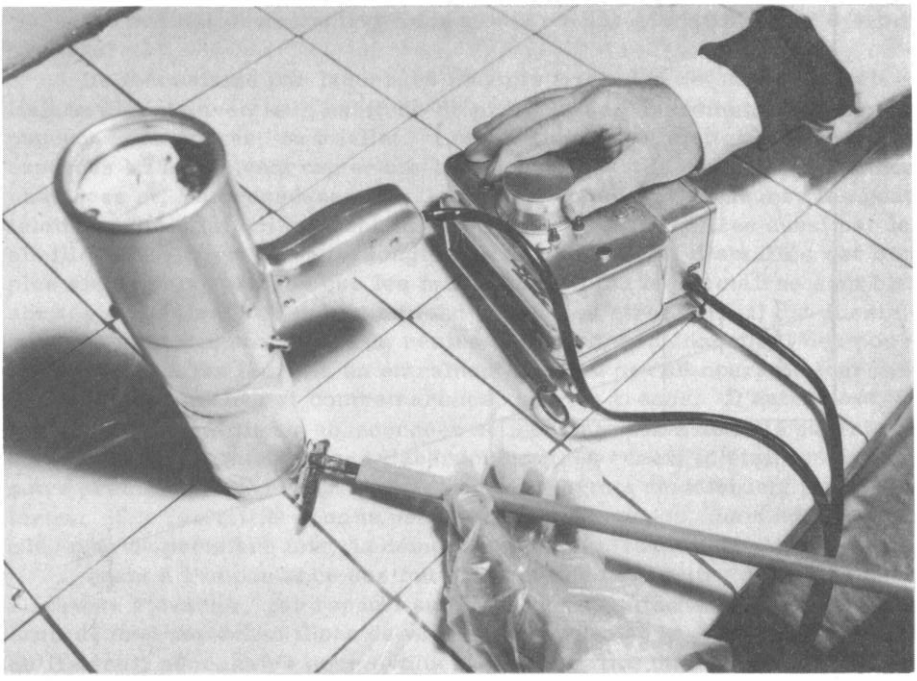

Figure 6

Marquage expérimental de sauterelles.

2. Eléments défavorables

Rendement du marquage faible: A peine 10% du traceur mis en œuvre semble avoir été absorbé par les insectes. Ceci est dû en partie à la mauvaise utilisation du traceur sur le blé. Ceci peut être, pense-t-on, fortement pallié par l'emploi de son, qui absorbe mieux le liquide traceur, comme nourriture marquée.

L'activité totale utilisée est élevée: Avec les détecteurs actuellement à notre disposition et le rendement de marquage précédent, il faudrait utiliser une trentaine de curies de ^{192}Ir pour le marquage de 500 000 individus, ce qui semble excessif. Encore faut-il noter qu'avec une telle activité, au bout de deux mois un insecte ne serait décelable qu'au contact même de l'appareil (au lieu de 50 cm réclamés par le demandeur).

Pour ces raisons entre autres, le marquage au Niger n'a pas eu lieu.

IV. ÉTUDES SUR LES GUÊPES*

Une étude assez semblable à celle entreprise avec les abeilles et concernant les échanges trophallactiques a été effectuée sur les guêpes Para-

* Etudes effectuées en collaboration avec M. Montagner, Faculté des sciences de Nancy.

vespula vulgaris, germanica et Dolichovespula media. Une dizaine d'individus ayant été nourris avec de l'or radioactif ont été re-introduits dans le guépier et nous ont servi à étudier la vitesse de transmission de la nourriture marquée dans le nid. Ces vitesses sont en général très grandes.

Ces études ont permis de dégager les principaux aspects des relations interindividuelles au cours des échanges alimentaires et d'éclaircir les rapports entre couvain et adultes.

1. Echanges entre ouvrières

Cette étude a été effectuée dans des cagettes à 3 compartiments, dont celui du milieu contenait seul la nourriture marquée; il était séparé des deux autres par une cloison perforée en plexiglass. Nous avons essentiellement montré que les ouvrières du compartiment central approvisionnent beaucoup mieux leurs sœurs que les étrangères. Ainsi, nous avons établi, pour deux séries d'expériences, que les pourcentages d'activité des ouvrières sans nourriture, rapportée à celle des nourrices du compartiment central, étaient, après 24 heures, de 45,1 et 43% pour les sœurs et de 19,4 et 22% pour les étrangères. D'autres expériences effectuées dans le même sens ont toujours confirmé ces résultats. Mais cette discrimination nutritionnelle disparaît avec le temps et n'est plus nettement décelable après 2 jours de vie commune.

2. Alimentation des mâles – leurs rapports avec les autres membres de la société

Les capacités de «self-alimentation» des mâles dépendent non seulement de leur âge, mais aussi du degré d'évolution du nid. Ainsi, les jeunes sont parfaitement capables de bien s'alimenter tout seuls, mais les adultes semblent refuser la prise de nourriture à une époque qui coïncide avec l'apparition des fondatrices filles ou la décroissance de l'activité sociale. Il semble donc qu'il se produise un arrêt dans l'alimentation des mâles, au moment des fécondations.

En outre, ils sont peu alimentés par les ouvrières. Nous avons montré que, dans le nid, ils se nourrissent essentiellement en provoquant les régurgitations des larves. Il semble donc que dans le nid les individus mâles vivent en parasites sur ces régurgitations larvaires, qu'ils savent obtenir facilement.

3. Les rélations alimentaires entre les ouvrières et le couvain

Les ouvrières nourrissent les larves en fonction de leur taille et de l'intégrité de leurs cellules. Lorsque des larves d'ouvrières et de fondatrices sont élevées en compétition, ces dernières sont toujours approvisionnées de façon plus abondante. Ajoutée à d'autres résultats cette constatation nous incline de plus en plus à penser que la quantité de nourriture donnée aux larves doit jouer un rôle essentiel dans leur différenciation en deux castes femelles.

V. ÉTUDE DE RÉPARTITION D'UN FONGICIDE*

Bien que ce travail sorte du cadre imposé par le titre de cet article, nous en dirons quelques mots car il s'inscrit dans le programme de ce colloque.

Dans les plantations de bananiers, des huiles minérales sont utilisées en tant que fongicide sous forme de diffusion lente de quelques centimètres cubes en un point déterminé de la plante. L'emploi d'une huile fongicide marquée au tritium a permis d'en étudier la distribution à l'intérieur du bananier, la vitesse d'écoulement dans différents tissus, et de comparer l'efficacité de différents modes d'injection.

La simple dissolution d'un élément marqué dans une huile n'a pas été utilisée car le métabolisme de l'élément dissous peut être très différent de celui de l'huile elle-même. La solution adoptée a donc été la fixation de tritium sur les doubles liaisons du squalène pour obtenir une huile comparable aux huiles minérales.

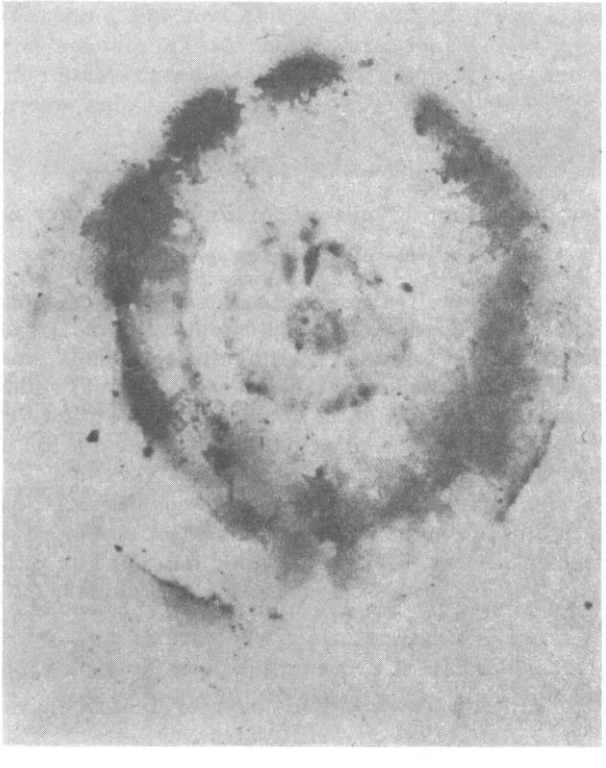

Figure 7

Autoradiographie d'une hampe de bananier marqué au squalène tritié.

* Etudes effectuées en collaboration avec MM. Cuille et Laville, Institut français de recherches fruitières d'Outre-Mer [10].

Après une étude en laboratoire démontrant la validité de la méthode proposée, une plantation de 30 bananiers, située à la Guadeloupe, a été traitée à raison de 50 mc de squalène tritié par arbre. On a fait varier les conditions d'injection (activité spécifique de l'huile, emplacement, durée et date de l'injection) et le traitement des arbres.

Périodiquement, des échantillons ont été prélevés sur les arbres dans différentes régions (limbes et nervures des feuilles, pseudotronc, bulbe et racine). Ces échantillons ont été brûlés, l'eau de combustion récupérée et comptée par scintillation liquide. L'activité de l'eau de la terre avoisinante a également été mesurée.

Simultanément, en laboratoire, de jeunes pousses ont été marquées à forte activité spécifique (250 mc/cm^3) et les diffusions étudiées par autoradiographie après congélation des échantillons dans l'azote liquide (fig. 7).

Les résultats obtenus montrent notamment que l'huile séjourne assez longtemps à l'endroit d'injection, puis se déplace préférentiellement vers les feuilles et les fruits, et qu'une faible fraction traverse le bulbe avant d'être éliminée par les racines.

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DISCUSSION

W. KLOFT: Your paper deals with work similar to what we have been doing ourselves since 1956, and it is very interesting to hear that you have also found that honey-bee drones are able to regurgitate crop food to workers. We first published our findings on this subject in 1958, but since they were not generally accepted, we repeated the study last year, using tracers and film analysis, and we found quite definitely that the drones are able to regurgitate. One great source of error lies in the distribution of radioactive drone excrement in the honey-bee community: as you know, drones can excrete their food two hours after intake and their radioactive excrement is taken up by the workers and spread through the colony very quickly. By using a special experimental set-up, however, we were able to exclude this source of error.

At the suggestion of Mr. Lecomte, the results of the experiments we carried out last year will be submitted to the journal Annales de l'abeille with a view to publication.

J. LECOMTE: I was most interested to learn that you had continued your work on this particular problem. The fact that similar phenomena have been observed in a different laboratory confirms our findings.

M. FERON: I was very interested in what the authors of this paper had to say on food exchange in social insects, and I think the same methods could also be applied to non-social insects. In the case of Diptera, the process of food exchange by frequent regurgitations is well known and the part it plays in the spread of pathogenic germs may be of importance both to man and animals and to the insects themselves. Perhaps this labelling method could provide useful information regarding such phenomena.

J. LECOMTE: That is true. However, the tracer study should then be coupled with other observations such as film analysis, designed to verify the absorption of regurgitated material and to eliminate the possibility of contamination from other sources.

R. CAVALLORO: In investigating the mechanism by which ants fix radioactive fall-out, did you measure the radioactive spectrum of the honeydew of aphids?

J. LECOMTE: No, but this has been done in Freiburg in Germany, using the honeydew taken up by bees from aphids.

R. CAVALLORO: What species of Acrididae did you use in your experiments?

J. LECOMTE: The Locusta migratoria.

W. KLOFT: We too have done field experiments on food distribution in ant colonies, especially in Formica polyctena and related species, and we obtained similar results with a spread of activity up to 200 m. However, in our field experiments on natural colonies of Formica polyctena with interspersed nests of Formica rufa, the Formica rufa did not take part in the exchange. I know this does not conflict with your own results, because we have done a lot of laboratory experiments which prove that there is good exchange between different species of the genus Formica. The explanation is to be found in the complex conditions existing in the field: relations between nests are influenced by the saturation of single nests, isolation, the distances involved, etc.

J. LECOMTE: We were indeed surprised at the exchange of food between different colonies of ants, but the determination of species was carried out in your own laboratories and I do not think we can question the results.

W. KLOFT: I did the sample analysis myself and the results are undoubtedly correct. Regarding your interpretation of the way in which ants have concentrated radioactive fall-out, I should like to say that I have done a lot of work on trophobiosis and have demonstrated that ants usually take food directly from the anus of aphids. Only in rare cases do they take honeydew from leaves, whereas the honey-bees have no direct relations with the aphids and always take the honeydew from leaves. I think, therefore, that in the case of predacious ant species the concentration of this fall-out may be due to the ingestion of leaf-eating insects.

J. LECOMTE: To reach some definite conclusions on this point, one would have to study in the same area and at the same time not only the radioactivity of the ants and bees but also that of the aphids, caterpillars, leaves,

etc. Our supposition is perhaps not correct, but we felt that honeydew taken from leaves probably plays an important role. The radioactivity spectrum of the honeydew collected by the honey-bee is very similar to what we observed in ants.

G.T. SCARASCIA: Have you any experimental evidence regarding the possibility that the resin of conifers may fix radioactive fall-out? Work carried out in Italy by the Agricultural Chemistry Station (Ministry of Agriculture) and by the National Commission for Nuclear Energy has shown no difference between the fall-out radioactivity of pines and that of other plants.

J. LECOMTE: We have no experimental evidence on the role of the resins. Our observations were limited to honeydew only, in view of the fact that resin is not collected by ants or bees.

J. HALBERSTADT: I noted in the paper that there is a difference in uptake of Au^{198} and Ir^{192} by bees, ants and grasshoppers. I also understand that there is a big difference in the location of these isotopes in the different insects after uptake. Could you give an explanation of these differences? Perhaps it is because gold is used in the form of a colloidal solution while iridium is used as a suspension and is therefore taken up poorly.

J. LECOMTE: The form in which the radioisotope is administered will certainly have some effect on the fixation rate and the biological half-life. We did not try to find out where the gold and the iridium are localized, but it appears that gold is mainly found in the walls of the digestive tract. We were interested in gold because of its ease of use, and we obtained good results with the colloidal form without appreciable difficulty. It was not necessary for us to try to improve the rate of fixation. As regards iridium, we have so far done only preliminary experiments. We have not gone very far in this field yet, and possibly by varying the method of administration we could improve the rate of fixation and the biological half-life, but this has not been studied as yet.

TRACER EXPERIMENTS ON FOOD EXCHANGE IN ANTS AND TERMITES

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Abstract — Résumé — Аннотация — Resumen

TRACER EXPERIMENTS ON FOOD EXCHANGE IN ANTS AND TERMITES. One of the most important aspects of social insects, such as ants, honey-bees and termites, is their tendency to trophallactic food exchanges, which can be studied well by the use of labelled food. In comparative studies we found the most rapid rate of exchange in honey-bees, but extreme differences were observed among the different subfamilies of ants, which must be taken in consideration in case of tracer field-experiments. The greatest tendency towards trophallactic exchanges was found in the subfamily Camponotinae. Ants of the genus Formica, especially Formica polyctena Först. and related polygenous and polycalous species known to be important factors in the preventive biological control of forest insect pests, were intensively analysed. It was found that the rate of food exchange within one nest is conditioned by temperature, time, number of individuals and saturation. Another question is whether food exchanges also take place between the different nests of ant-colonies of the same species. By labelling single nests with radioactive food we were able to find intensive food exchanges with different nests in the colony, up to distances of 200 m. We obtained similar results in three different colonies and in different years. The existence of such long-range food exchanges is very important for the estimation of the regulating effect on insect pests of useful Formica species, since these exchanges prevent the quick saturation of any nest through local mass-infestation of insects in their predating area. It was shown by the tracer experiments that the collected food flows in most of the surrounding nests of the ant-colony: thus colonies of these useful wood-ants act as a complex system with high ecological effectiveness.

Using labelled food, we studied in termites (Kalotermes flavicollis Fabr.) which stages and castes are capable of direct feeding or are receptors of stomodeally or proctodeally given trophallactic food. Pseudoworkers are most effective. We also attempted to use tracer methods to explain the greater longevity and greater aggressiveness of termites when in groups than as single individuals. Pseudoworkers of Kalotermes flavicollis were labelled with I^{131}. After feeding, some of these labelled termites were caged individually, while other groups were formed which also included unlabelled individuals. By measuring the effective half-life and calculating the biological half-life, which primarily depends on the rate of excretion, we found that the tracer remained longer in groups - when the group is viewed as one unit - than in isolated individuals. The result can be explained by the measured trophallactic exchanges of food and repeated circulation among individual members comprising a group. Similar results were obtained with two ant-species from different subfamilies. The greater economy in the use of food and other substances may be a contributory factor to the "group-effect" in social insects.

Special problems of comparable measuring techniques for living single individuals and insect groups are dealt with.

EMPLOI DES RADIOINDICATEURS DANS L'ÉTUDE DE LA TROPHALLAXIS CHEZ LES FOURMIS ET LES TERMITES. Un aspect particulièrement important de la vie des insectes sociaux comme les fourmis, les abeilles et les termites est la tendance à la trophallaxis, qu'il est facile d'étudier au moyen de nourriture marquée. Des études comparatives ont montré que la trophallaxis est le plus rapide chez les abeilles, mais qu'elle varie considérablement d'une sous-famille de fourmis à l'autre, facteur qui doit être pris en considération dans les expériences faites sur le terrain à l'aide de radioindicateurs. La plus forte tendance à la trophallaxis a été relevée chez les individus de la sous-famille Camponotinae. Les auteurs ont étudié tout particulièrement les fourmis du genus Formica, en particulier la Formica polyctena Först (fourmi rousse) et les espèces polygènes et pluridomes apparentées, qui représentent un facteur biologique important dans la lutte contre les insectes nuisibles aux forêts. Les auteurs ont observé que la trophallaxis à l'intérieur d'une fourmilière est conditionnée par la température, l'époque, le nombre d'individus et la saturation. Un autre problème est de savoir s'il y a également trophallaxis entre différentes fourmilières de colonies appartenant à la même

espèce. En introduisant de la nourriture marquée dans diverses fourmilières, les auteurs ont pu détecter des échanges intensifs entre des fourmilières séparées par des distances allant jusqu'à 200 m. Ils ont obtenu des résultats semblables pour trois colonies différentes, au cours de plusieurs années. Cette trophallaxis à grande distance a beaucoup d'importance pour l'évaluation du rôle des espèces Formica dans la lutte contre les insectes nuisibles; elle empêche toute fourmilière d'être rapidement saturée par l'infestation massive d'insectes dans la zone d'activité des fourmis: les expériences faites à l'aide de radioindicateurs montrent que la nourriture accumulée est échangée entre la plupart des fourmilières voisines. Ainsi, les colonies de fourmis rousses utiles constituent un complexe qui peut jouer un rôle important sur le plan écologique.

A l'aide de nourriture marquée, les auteurs ont étudié quelles formes et castes de termites (Kalotermes flavicollis Fabr.) interviennent par voie buccale ou anale dans la trophallaxis. Les résultats les plus intéressants ont été enregistrés chez les pseudo-ouvriers. Les auteurs se sont aussi efforcés d'étudier, à l'aide de radio-indicateurs, les causes pour lesquelles la longévité est plus élevée et l'agressivité plus forte chez les termites en groupes que chez les termites isolés. Ils ont marqué des pseudo-ouvriers de Kalotermes flavicollis à l'iode-131. Après les avoir nourris, ils ont placé dans des cages individuelles un certain nombre des individus marqués et ils ont formé en même temps d'autres groupes qui comprenaient aussi des individus non marqués. En mesurant la période effective et en calculant la période biologique, qui dépend essentiellement du taux d'excrétion, ils ont constaté que le radioindicateur persistait plus longtemps chez les individus en groupes — le groupe étant considéré comme l'unité — que chez les individus isolés. Ce résultat peut être attribué à la trophallaxis et à la circulation répétée entre les individus du groupe. Les auteurs ont obtenu des résultats semblables pour deux espèces de fourmis appartenant à des sous-familles différentes. Il se peut qu'une plus grande économie dans l'utilisation de la nourriture et d'autres substances contribue à «l'effet de groupe» chez les insectes sociaux.

Les auteurs étudient les problèmes particuliers que pose la mise au point de méthodes de mesure comparables pour les insectes isolés et les groupes d'insectes.

ИЗУЧЕНИЕ С ПОМОЩЬЮ ИНДИКАТОРОВ ОБМЕНА ПИЩЕЙ У МУРАВЬЕВ И ТЕРМИТОВ. Самым важным для таких общественных насекомых, как муравьи, медоносные пчелы и термиты является их стремление к трофоллактическому обмену пищей, который может быть хорошо изучен с помощью меченной радиоактивными веществами пищи. При сравнительном изучении самая высокая скорость обмена была обнаружена у медоносных пчел, в то время как у различных подсемейств муравьев она была чрезвычайно разнообразной, что должно быть учтено при проведении полевых экспериментов с применением индикаторов. Самое сильное стремление к трофоллактическому обмену мы обнаружили у подсемейства Camponotinae. Усиленно изучались муравьи Genus Formica, особенно Formica polyctena (Först.), и родственные полигенные и polycalous виды, которые играют важную роль в предупредительной биологической борьбе с насекомыми-вредителями леса. Установлено, что скорость обмена пищей в одном муравейнике обусловлена температурой, временем, количеством особей и насыщением. Другой проблемой является вопрос о том, существует ли обмен пищей между различными муравейниками колонии муравьев одного вида. Пометив отдельные муравейники с помощью внесения радиоактивной пищи, мы смогли обнаружить интенсивный обмен пищей между различными муравейниками колонии, отстоящими друг от друга на расстояние до 200 м. Аналогичные результаты мы получили для трех различных колоний в различные годы. Существование такого дальнего обмена пищей является очень важным для оценки регулирующего действия полезных видов муравьев в отношении насекомых-вредителей. Дело в том, что обмен препятствует быстрому насыщению популяции любого муравейника вследствие местной массовой инвазии насекомых в их предыдущем гнезде. Как показали эксперименты с применением индикаторов, собранная пища попадает в большинство окружающих муравейников колонии муравьев. Поэтому колонии этих полезных лесных муравьев действуют как сложная система с высокой экологической эффективностью.

С помощью меченной радиоактивными веществами пищи мы изучили, какие категории и касты термитов (Kalotermes flavicollis Fabr.) способны к прямому питанию и какие питаются трофоллактической пищей, отрыгиваемой кормильцами изо рта в рот или получаемой из прямой кишки последних. Наиболее эффективными являются псевдорабочие. Мы также пытались объяснить с помощью индикаторных методов причины большей продолжительности жизни и большей агрессивности групп термитов по сравнению с отдельными изолированными особями. Псевдорабочие Kalotermes flavicollis метились J^{131}. После кормления некоторые из меченых термитов были изолированы поодиночке, а из других термитов образованы группы, включающие также неменеченых особей. Измерив эффективный период полураспада и рассчитав биологический полупериод, который прежде всего зависит от скорости выделения, мы обнаружили, что индикатор дольше остается в группе (когда группа рассматривается как единое це-

лое), чем у изолированных особей. Это можно объяснить измеренным трофолактическим обменом пищей и повторной циркуляцией среди отдельных особей, составляющих группу. Аналогичные результаты мы получили с двумя группами муравьев, относящимися к различным подсемействам. Большая экономия в потреблении пищи и других веществ может быть фактором, способствующим "групповым действиям" общественных насекомых.

Рассматриваются специальные проблемы методов сравнительных измерений живущих отдельно особей и групп насекомых.

EMPLEO DE INDICADORES RADIACTIVOS PARA ESTUDIAR LA TROFALAXIA EN LAS HORMIGAS Y LOS TERMITES. En los insectos sociales – hormigas, abejas y termites – es de suma importancia la tendencia a la trofalaxia, que puede estudiarse bien mediante alimentos marcados. Los autores de la memoria han podido comprobar en estudios comparativos que la trofalaxia es más rápida en las abejas, pero han observado diferencias considerables entre las diversas subfamilias de hormigas, lo que debe tenerse en cuenta cuando se efectúan experimentos con indicadores. La subfamilia Camponotinae es la que tiene una tendencia más marcada a la trofalaxia. Los autores estudiaron hormigas del Genus Formica, en particular la Formica polyctena Först, y otras especies poligénicas afines que desempeñan un importante papel en la lucha biológica preventiva contra las plagas de insectos forestales. La trofalaxia dentro de un nido está condicionada por la temperatura, el tiempo, el número de individuos y la saturación. Otro problema es el de saber si existe también trofalaxia entre diferente nidos de colonias de hormigas de la misma especie. Introduciendo en algunos nidos alimentos marcados, los autores observaron un considerable intercambio de alimentos entre los nidos de la colonia hasta 200 metros de distancia. Lograron resultados análogos en tres colonias diferentes y en años distintos. La trofalaxia a grandes distancias es un factor muy importante para evaluar la acción destructiva que ejercen las especies útiles de Formica sobre los insectos nocivos, ya que impide la rápida saturación de los nidos por una infestación local masiva de insectos en la zona de merodeo. Los experimentos con indicadores muestran que los alimentos acumulados pasan a la mayoría de los nidos vecinos de las colonias de hormigas. Por tanto, las colonias de la Formica rufa actúan como un sistema complejo de gran eficacia ecológica.

Con ayuda de alimentos marcados, los autores estudiaron en los termites (Kalotermes flavicollis Fabr.) qué fases y castas intervienen en la trofalaxia bucal o anal. Los seudoobreros son los más eficaces. Utilizando indicadores radiactivos, los autores trataron de descubrir por qué razón los termites en grupo son más agresivos y tienen mayor longevidad que los termites solitarios. Los seudoobreros fueron marcados con ^{131}I. Después de alimentarlos, algunos de estos termites marcados fueron encerrados en cajas por separado, y al mismo tiempo se formaron otros grupos que comprendían termites no marcados. Midiendo el período eficaz y calculando el período biológico, que depende ante todo del índice de excreción, los autores observaron que el indicador permanecía más tiempo en los individuos en grupo – considerando el grupo como una unidad – que en los individuos aislados. Este resultado puede explicarse por la trofalaxia y una circulación frecuente entre los individuos del grupo. Se obtuvieron resultados análogos con dos especies de hormigas de dos subfamilias distintas. La mayor economía en el empleo de alimentos y otras sustancias puede contribuir al «efecto de grupo» en los insectos sociales.

En la memoria se examinan algunos problemas especiales de las técnicas de medición comparables para grupos de insectos e insectos aislados.

INTRODUCTION

The exchange of food and other substances between individuals of the colony is one of the most important integrative mechanisms among social insects. Especially among the highly organized social insects forming insect-states, the labile equilibrium between one or a few reproductives and a very large number of non-reproductives possessing a more or less latent sexual potentiality (worker ants and honey-bees) or potentiality for development into reproductives (termite larvae) is maintained primarily through such mutual exchanges. Social food exchanges are among the preconditions of division of labour, of brood-rearing in the optimal ecological conditions of the nest,

and of any other development into higher forms of social life among insects. They may be also a means of communication between individuals. The study of these exchanges is not only of great scientific interest but also of practical interest, since many of the social insects have great economic importance in agriculture and forestry. The use of radioactive isotopes as tracers offers excellent possibilities for the basic study of these processes. In 1952, NIXON and RIBBANDS [1] introduced the tracer method into the study of food transmission within the honey-bee community; we followed in our laboratory with a series of papers from 1956 on concerning ants [2-15, 37-38], termites [4, 9, 16, 17, 18] and honey-bees [4, 9, 19]. The present paper outlines the results of our tracer studies with special reference to unpublished experiments on ants and termites.

A. FOOD EXCHANGE IN ANTS

1. Methods

In the experiments, P^{32} and I^{131} were used as tracers. They were administered to the ants in honey-water, saccharose solutions or pure water [20] at specific activities of 1 μc/ml up to 1 mc/ml, depending on the specific experimental purposes. The liquid food was offered on small glass dishes or the ants were individually supplied through glass pipettes, and the greatest possible care was taken to prevent external contamination. If necessary we decontaminated the radioactively fed ants that were to act as food donors by a special method, bathing them first in watery solutions of the inactive compound (sodium phosphate or sodium iodide) and then in pure water. They were dried quickly by letting them run about on layers of soft absorbent cellulose, which were changed several times. Measurements were carried out with G-M end-window counting tubes (window thickness 1.2-1-4 mg/cm²) or with a scintillation counter, both in conjunction with electronic scalers and (if necessary) with automatic sample-changers.* Live ants were enclosed for counting in small measuring cages consisting of a ring of glass covered by a thin membrane. The diameter of these rings was adapted to the size of the ant-species and ensured the same geometrical arrangement in comparative studies.

2. Food exchange among worker ants

The most intensive studies were carried out with ants of the genus Formica, especially the Formica rufa group (F. rufa L., F. polyctena Foerst., F. pratensis Retz. (syn. F. nigricans Em.), F. cordieri Bondr.). If one freshly fed worker is placed as donor within a group of hungry individuals, the donor supports through direct feeding (regurgitation of crop-content) the major part of the food ingested up to 6-8 ☿☿ [2, 3, 7]. Between 7° and 35°C the percentage of transmitted crop activity is the same, but the time required depends on the temperature [12]. The primary recipients of food act as

* Made by Frieseke & Hoepfner, Erlangen

secondary donors and so on, and the food exchanges continue to produce a multilateral food chain. In this way up to 80 ☿☿ can take part of the content of one crop. With honey-bee workers we found such distributions from one crop-content only up to 50 individuals, since the individual food consumption crop-content only up to 50 individuals, since the individual food consumption (crop → midgut → resorption) is higher than in ants. The distribution rate is influenced by time, temperature and the number of individuals within the group. Under conditions of constant temperature, the temporal course of food distribution follows an exponential function, so that after some time all individuals, up to a maximum of about 80, have been reached. Food exchange starts at 5-7°C, reaches its highest frequency between 25 and 30°C and then falls until the limit of 45-50°C is attained [12]. This optimal temperature is identical with the temperature in the centre of the nest-hill. The greater the group the more individuals can take part in the distribution of radioactivity. By measuring not only the number of radioactive ants but also the activity rate of each individual, we find that at first the distribution is very heterogeneous: some individuals have very high activities while others show extremely low activities. Through often repeated secondary regurgitations, the distribution reaches after some time a statistical equalization, following a Gauss "normal distribution" (Fig. 1) [4, 6, 7]. Over a longer period

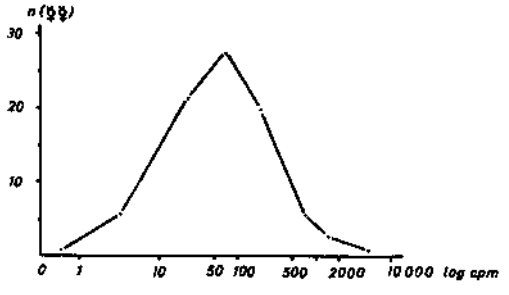

Fig. 1

Distribution of radioactive food within worker-groups of Formica polyctena Foerst.
The graph summarizes two experiments with groups of 50 workers (☿☿) each to which was given a single radioactive worker as food-donor for 20 h at 25°C. Counts per minute, showing the amount of ingested food, are plotted against number of individuals receiving a certain amount of this food.

of time, it is found that the "normal distribution" does not persist but gives way to a single-peak distribution with preference for progressively smaller exchange units. In this way each individual of the group or colony is assured of an adequate food supply. An increase in the number of donors results in a corresponding increase in the speed of distribution. We have not yet done such detailed experiments in honey-bee groups, but we can state that the individual food exchanges through regurgitation from proboscis to proboscis take place more quickly among bees than among ants.

In comparative tracer studies with more than 20 species of ant we found extreme differences between the four subfamilies available in our region and also between biological different species within the same subfamily. The

highest tendency to trophallactic exchanges was found in the subfamily Camponotinae (genera Camponotus, Formica, Lasius), although there is one species of this subfamily that has been shown in our laboratory to act only as receptor of labelled food [7, 13]: this is Polyergus rufescens Latr., the Amazon ant, which keeps in its nest workers of other species (G. Serviformica) as slaves responsible for food supply and distribution. While the workers of the Amazon ant are still able to ingest some food directly, other social-parasitic species have lost the capacity to do this, as has been proved by tracer techniques [7]. In tracer experiments with colonies of Epimyrma gösswaldi Men. living on a social-parasitic basis with colonies of the genus Leptothorax, it was possible to demonstrate a significant preference on the part of the parasitic ants for food supply through donors of the host ant (L. unifasciatus in our case). This result points the way towards one possible explanation for the process of selective evolution from normal independent ant species through social parasites of varying degrees up to the end-type, the workerless ant. Workers of the subfamily Monerinae have only very weak food exchanges among themselves; the individuals seem to be self-supporting. One species of Dolichoderinae that was studied showed relatively good food exchanges. Great differences were found among Myrmicinae; this agrees with the results of WILSON & EISHER [21], who carried out tracer studies with five American species. A high trophallactic tendency among workers seems, according to our experiments, to be correlated with trophobiotic habits. The demonstrated differences must be taken into consideration in field tracer experiments with ants. In the light of our experience we recommend that some distribution experiments should first be carried out among worker groups before starting with species not yet studied.

3. Food distribution among different castes and stages

Old queens, young queens before and after mating and males of F. polyctena feed directly on radioactive honey-water in amounts similar to those for workers. We also proved the capacity of sexuals to distribute food by regurgitation in the following experimental combinations: ♀♀⁺; ♀♀, ♀♀⁺; ♂♂, ♀♀⁺; ♀♀, ♂♂⁺; ♂♂, ♂♂⁺; ♀♀ and ♂♂⁺; ♀♀. In all cases the experiments were positive, the rate of distribution of ♀♀ being similar to that of workers, while that of males was smaller. The most notable observation was the capacity of Formica males for food distribution. We obtained similar results with Lasius and especially with Camponotus ligniperda ♂♂ [40]. These males remain for about nine months in the imaginal stage in their mother's nest and not only distribute food to other ♂♂, ♀♀ and ♀♀ but even transfer radioactivity to larvae. This trophallactic tendency is less marked when the overwintering and the subsequent mating phases are reached. Using tracer methods, we obtained similar results with the drones of honey-bees which are undoubtedly also able to regurgitate and distribute crop food [4, 9, 19]. A source of error are radioactive excrements which are fed and distributed through workers. Mated queens of Apis mellifica distribute radioactive crop substances among workers. In further experiments that we carried our with newly founded colonies of Lasius niger L., the queen showed a

decreasing tendency to supply larvae with radioactive food through herself after the first imaginal workers (pygmies) had hatched: The pygmean workers and the queen acted mutually as both donors and receptors of food. The larvae of the species Camponotus ligniperda L. and C. herculeanus L. have during the overwintering phase a growth diapause which under laboratory conditions is maintained even at relatively high temperatures. They do not normally receive food but at higher temperatures they were supplied with small amounts of labelled food [14].

In agreement with WILSON & EISNER [21], we often found that queens in relatively large groups or colonies showed no preference for the supply of radioactive crop food introduced through foraging ants. By the application of P^{32}, it was found that the reason was transfer of glandular substances. These substances cannot be labelled until the ingested radiophosphorus is assimilated throughout the body. This process begins 1-2 h after feeding and takes 24-48 h, depending on the temperature [4, 7].

In special experiments we were able to distinguish between primary crop food and transfer of glandular secretions [15]. Radioactively fed workers were isolated for 48 hr, after which they were allowed to empty their crop through repeated regurgitations. Then they were fed several times with inactive food solution which they were permitted to regurgitate to feed further hungry workers. By measuring these receptors we were able to determine the point at which the crop was completely cleared of the primary crop activity. After some time, especially in Spring, the crops of such workers acquired secondary radioactivity due to secretion of pharyngeal glands. Since these glands open into the pharynx and have no reservoirs of their own, their secretions are swallowed and may be stored in the crop, which thus acts as a kind of reservoir for the pharyngeal glands. This secondary crop content is regurgitated, especially during the early springtime, more or less pure as a special food for queens and for rearing the sexual brood. It seems to have caste-determining or differentiating effects on the female larvae [38]. After external foraging begins, the external food is mixed with gland secretions through repeated regurgitations and thus becomes more or less ameliorated before reaching brood or queens. Thus we have an explanation for the often very weak transfer of radioactivity to queen ants in colonies.

4. Field experiments on food exchange among different nests of Formica colonies

As shown in the previous sections we have intensively analysed, using tracer methods, the trophallactic exchanges of ants of the genus Formica, especially Formica polyctena Foerst. and related species. These ants, especially polygynous and polycalous species, are known to be important factors in the biological prevention and control of forest insect pests [22]. An important question is whether food exchanges also take place between the different nests of ant colonies of the same species. It is known that natural colonies of the polygynous F. polyctena develop by forming daughter nests. It is apparent from the occasional exchanges of brood, workers and queens that close relations at first continue to exist between mother-nests and daughter-nests. Later the nests seem to be more or less independent except

for periodical migrations between nests which are at present under investigation. Our special problem was to find out whether exchanges of food take place under normal conditions, i.e. in the absence of visible migrations and exchanges of population. The tracer method was first used for problems of this kind by KANNOWSKI [23] who employed labelled food to determine whether each of the numerous nest-mounds of the subterranean ant Lasius minutus Emery in a swamp was an independent unit or not. He found that usually four or five neighbouring mounds seemed to form a colony with close feeding relations. Similar studies were carried out by MORTREUIL and BRADER [24] in African pineapple plantations in order to elucidate the dimensions of ant nests, their underground relations and their associations with harmful coccids. As was pointed out during the discussion of Mortreuil and Brader's paper at the Bombay Symposium (1960) [25], we have carried out this type of ecological tracer study for several years with interesting results. Beginning in 1959 and continuing through 1961/62 we labelled single nests of F. polyctena in one natural and two artificially founded colonies [22].

The tracer (P^{32} and/or I^{131}) was given in honey-water solutions. To prevent external contamination we offered the food in small special glass or plastic vessels which were introduced into the nest-mounds; this prevented the contamination of other insects. During the first experiments we gave only small activities, e.g., in a natural colony, 1 mc I^{131} dissolved in 10 ml honey-water. The activity was introduced in a single food-vessel into the south-west sector of nest-mound A (31.5.1959, 11 a.m.). After 46 h we took several samples of 20 ♀♀ each out of all 5 nests, which were counted in the laboratory with a scintillation counter. Secondary radioactivity was found in ants from nests C and D (Fig. 2); though weak, it was significantly different from the background activity and from the activity of ants from untreated nests in the same area. Surprisingly, we detected no transfer of radioactive food into nest B and the very near newly founded daughter-colony a. We suggest that a single food vessel was insufficient for labelling the whole population of the nest. Meanwhile, CHAUVIN, COURTOIS and LECOMTE, who independently from us did similar work with Formica [26, 27],

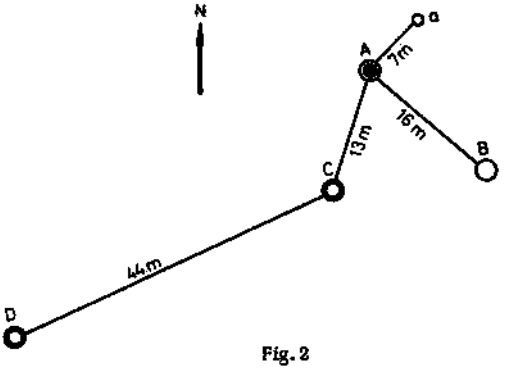

Fig. 2

Distribution of radioactive food among nests of a natural colony of Formica polyctena Foerst.
◉ primary labelled nest, labelled with 1 mc I^{131}
● nest showing secondary radioactivity after 46 h
○ nest inactive after 46 h

showed in their second paper similar sectorial preferences of food transmission within ant-nests. In all later experiments we divided the tracer into 6-10 vessels which were well distributed over the primary labelled nest-mound.

Further experiments were done in artificially founded colonies [22]. One artificially founded nest (No. 59/1) showed two months after foundation migrations of changing intensity over a distance of 75 m to a secondary daughter-nest (No. 59/3a) which had been established through the artifically founded nest No. 59/3. Nest 59/1 was labelled with 0.12 mc P^{32} + 0.2 mc I^{131} in 4 ml honey-water (5. 6. 1959). After 96 h samples of 25 ♀♀ each were taken from 59/1, 59/3a and two other old-established nests (No. 104, No. 5) 70 m away from the primary-labelled 59/1. They were counted with G-M tube and scintillation counter in the laboratory. The results can be seen in Table I, which gives the counts per minute, including statistical error, obtained with two different counting methods. The transmission of radioactivity was positive only to nest 59/3a, in which the level of radioactivity was about two thirds of the counts in 59/1. The transmission may have been conditioned in this case by mixing of the nest population as well as by food exchange.

TABLE I

TRANSMISSION OF RADIOACTIVITY
AMONG DIFFERENT NESTS OF F. POLYCTENA
Nest No. 59/1 primary-labelled with P^{32}+ I^{131},
transfer positive only to nest 59/3a.

Sample	G-M tube (counts/min)	Scintillation counter (counts/min)
Background chamber	19.2 ± 1.8 18.9 ± 1.9	133.7 ± 3.6 138.0 ± 8
25 ♀♀ from nest 59/1	154 ± 8 169 ± 7	210 ± 8 195 ± 6.9
25 ♀♀ from nest 59/39	100 ± 7 89 ± 6.2	163 ± 8 174 ± 8.2
25 ♀♀ from nest 104	19.8 ± 2 18.6 ± 1.8	143 ± 6 141 ± 6
25 ♀♀ from nest 5	18.9 ± 1.7 19.7 ± 2	139 ± 7 138 ± 8

The next experiment, carried out with an artificially enlarged colony of F. polyctena, showed long-range transmissions of radioactivity over distances up to 200 m. The primary-labelled nest No. 7 (Fig. 3) received 10 mc I^{131} in 30 ml honey-water (12 June 1959). Countings of samples consisting of 100 ♀♀ each and taken 72 h after primary labelling were carried out with the scintillation counter in the laboratory. Seven of 13 nests carried significant secondary labelling. Visible connection, through migration of

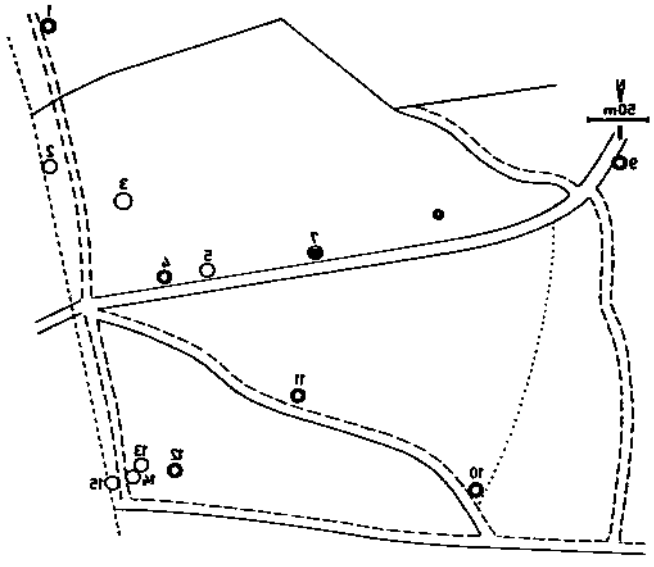

Fig. 3

Transmission of radioactive food from a single primary labelled nest to others

- ● primary labelled nest
- ◘ nest with secondary radioactivity after 72 h
- ○ nest inactive after 72 h
- • small unnumbered daughter nest, radioactive after 72 h

workers in both directions, existed only between No. 7 and No. 9, including the small unnumbered daughter nest.

During the same summer season we started a twice-repeated experiment in an artificially founded colony of F. polyctena in an area containing single nests of a natural population of F. rufa. Radioactivity was transmitted during June into most surrounding nests up to a distance of 70 m in a direct line, except for one nest of F. rufa which was at the shortest distance (30 m) from the primary-labelled nest of F. polyctena (the tracer dosage was the same as in the experiment described earlier). In the surroundings of the primary-labelled nest were three newly founded (two months old) artificial daughter nests. These did not partake of the radioactive food. But when we repeated the experiment under the same conditions three months later (4 September 1959) we found radioactivity in the daughter nests too. Especially this last result is very important since it shows that new daughter colonies, founded with ants of the same species but strange origin, will be integrated in the colony-system in the course of a few months. The experiment also confirms an observation published in 1940 [39] that ants of artificially founded daughter nests established in the mother nest's area migrate back, since they have too close social relations. Daughter nests of the same species but strange origin need some months to develop such relations and become well-established in the meantime.

The results of these field tracer experiments are of great importance in evaluating the regulating effect of useful Formica species on insect

pests [28], since these exchanges prevent the quick saturation of any nest by local mass infestation by insects in its predating area. The food collected flows, as shown by the tracer experiments, into most of the surrounding nests of the ant colony. Thus colonies of these useful wood-ants are acting as a complex predating system with high ecological effectiveness. In this connection it should be mentioned that we also have tested the distribution of radioactive caterpillars (activated by feeding on radioactive leaves), which were taken as living prey. Though the specific activity of the caterpillars was not very high, we observed good transmission within small colonies of F. polyctena under laboratory conditions.

We do not know exactly how the exchanges of radioactivity among different nests operate. Mixing of populations through migrations in both directions may play a part, as well as food transmission through regurgitation between workers of different nests meeting outside the nests. Both events can be observed, but may be different from case to case under the complex natural conditions. They should be analysed by labelling the ants with a combination of externally visible colours and radioactive food.

In some further experiments we tried to determine whether the food transmission operates better among artificially founded nests of the same origin than among these of different origin. Since these experiments were carried out at the beginning of the overwintering phase we found only weak transmissions of radioactivity between nests (1-15 October 1962). However these experiments yielded another very interesting result: during the overwintering phase the population stores food reserves in the hypertrophied fat-bodies of a part of the population [12, 20], especially of young workers, as has been suggested [20].

These "fat" individuals with swollen gasters are in the depth of the nests. During overwintering their behaviour pattern changes: their metabolism is reduced and they do not accept radioactive food [12]. Though they are already in the deep parts of the nests, they show a significant preference for supply with radioactive food offered at the nest surface. The storing individuals seem to be filled up as well as possible during this period of preparation for the winter.

For the last two years we have been using a portable counter independent of current mains. The apparatus was developed in conjunction with an engineering bureau * for our special purposes. The apparatus is fully transistorized and equipped with Ni-Fe batteries and a 220-V AC generator. It supplies the G-M tube with direct current up to 1800 V and the counter (which works electronically in two decades and mechanically in three) with 220-V alternating current (see Fig. 4). A start-stop device with time-intervals of 1 to 10 min is also fitted.

B. FOOD EXCHANGE IN TERMITES

Since termites are hemimetabolic insects, all stages - apart from the egg - are mobile and have well-developed mouth-parts, though specialized in the case of soldiers. For that reason it is more difficult to obtain an

* Radiomed (Firma Erich Jaeger), Würzburg

Fig. 4

Portable counter for field measurements

insight into the food relations among the members of a termite-colony than with social Hymenoptera. However, the tracer method offers excellent possibilities of studying these problems in termites. We began work of this type in 1958 in our laboratory with the drywood termite Kalotermes flavicollis Fabr. [4, 16, 9, 17, 18]. This species is very suitable as a laboratory termite for testing the termite-resistance of materials [29, 30, 17, 18].

It first had to be determined which stages were capable of direct uptake of foodstuffs. The termites were carefully separated into the different stages. We offered them as food filter paper soaked with P^{32} solution (specific activity 1 mc/ml). To avoid contamination, the termites were forced to eat through a small hole (diameter corresponding to the head width of the state studied) in a plastic foil stretched over the radioactive filter-paper. The experiments showed that larvae of the first stage, soldiers and preimagines (short interstage between second nymph and adult), and also alate termites never feed directly. Larvae of the second stage take up only very small amounts. The most active feeders are larvae of the third, fourth and fifth stages and pseudergates. In relation to their size, the two last-mentioned stages eat the largest amount of radioactive paper, thus demonstrating their suitability for test purposes [17]. Nymphs of both stages are also very active feeders, but they need a pre-adaption time of two days. In a similar way imaginal sexuals, more than three years old, regained the capacity to feed directly after an isolation time of 65-75 h.

Food exchange among individuals of K. flavicollis takes place relatively slowly. Putting radiolabelled termites at 27°C into normal colonies (ratio

labelled: unlabelled = 1 : 10), we found that after 12 h 40%, after 20 h 70%, and after 35 h 100% of the individuals were radioactive. The older larvae and young nymphs, especially the pseudergates, play the main part as donors, while young larvae, older nymphs, sexuals and soldiers are receptors of food. Moulting individuals do not feed two days before and two days after this event [18]. The exchange of food and other substances between termites takes place both stomodeally (i.e. by mouth-to-mouth feeding of regurgitated crop content or glandular secretions) or proctodeally (by feeding of excreted rectal content with a more or less fluid consistency)* Such protodeal exchanges are known also among ants of the subfamily Dolichoderinae [31] and tracer methods enabled them to be demonstrated also in larvae of Myrmicinae [7].

C. STUDIES ON THE EXCRETION OF I^{131} IN ANTS AND TERMITES: COMPARISON OF SINGLE ISOLATED INDIVIDUALS WITH INDIVIDUALS IN A GROUP

As shown in the previous sections social insects have a high tendency to trophallactic changes of food and other substances, e.g. cuticularly excreted P^{32} [11, 32]. We supposed that, as a consequence of such repeated transmissions among individuals, the utilization of substances in groups might be more economic than in single individuals. A comparison of measurements of the biological half-life of a tracer in single isolated individuals and in individuals within a group seemed to be a convenient method for this problem. We studied the biological half-life of a γ-emitter, I^{131}, because as a result of changing body distribution and body absorption of β-particles precise determinations of the real effective half-life through repeated measurements of living insects are nearly impossible with β-detecting counters [32], unless the insects are very small and have thin cuticles, e.g. aphids [33]. However, all these complications are greatly reduced if γ-sources are used as tracers and the measurements are made with scintillation counters.

Pseudo-workers of the termite K. flavicollis and workers of the ant Formica pratensis Retz. (syn. Formica nigricans Em.) (Camponotinae) and Myrmica scabrinodis Nyl. (Myrmicinae) were fed with I^{131} by the methods described above (termites with labelled filter paper, ants with labelled honeywater). After the insects had eaten, the labelled ones were separated and kept in containers which were changed daily to avoid contamination through excreted radioactivity, either singly or in groups with unlabelled individuals of their nest (K. flavicollis: 1 labelled + 4 unlabelled; ants: 1 labelled + 5-15 unlabelled) under constant conditions of temperature (27°C) and air humidity (98% r.h.). During the experimental time fresh inactive food was always available.

Both the initial and the subsequent measurements of the radioactivity of the insects were taken with the help of a scintillation counter under the same geometric and other physical conditions. Before each measurement the background activity was first measured and subsequently subtracted from the measurements of the radioactivity of the insects. The insects were kept

* Shortly before the opening of the Symposium, a paper by McMAHAN was received, dealing with similar tracer studies of termite feeding relationships carried out with the drywood termite Cryptotermes brevis Walker [41].

under the scintillation counter enclosed in a small glass ring. Its diameter was different depending on whether single insects or a group of insects as a single unit were to be measured. It is interesting to note that the sum of the measurements of the individual insects composing a group was found statistically to be the same as the measurement of the radioactivity of the group when viewed as a unit.

The biological half-life (T_{biol}), which primarily depends on rate of excretion, was calculated from measured effective half-life (T_{eff}) and the known physical half-life (T_{phys}) by means of the following relationship:

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}},$$

The results are given in Table II. More details will follow in special papers [10, 34].

TABLE II

BIOLOGICAL HALF-LIFE (T_{biol}) OF I^{131} IN ISOLATED INDIVIDUALS AND IN GROUPS OF TERMITE PSEUDOWORKERS AND WORKER ANTS

	T_{biol} (h)		No. of experiments	Statistical constant (t)*	Probability
	Isolated single individuals	Groups			
K. flavicollis	354.3	580.9	21	2.84	0.02
F. pratensis (syn. F. nigricans)	19.3	43.9	24	2.15	0.05
M. scabrinodis	83.0	191.2	14	2.21	0.05

* Student's t-test (1928)

The summarized results show that the biological half-life is shorter in isolated individuals than in groups. Since the measurements of the single individuals clearly showed the repeated interindividual exchanges of radioactivity among individuals, it became apparent that the prolonged T_{biol} was a consequence of this circulation. The greater economy in the use of food and other substances may be a contributory factor to the "group-effect" by which GRASSE and his group [35, 36] tried to explain the higher longevity and greater vigour of social insects in groups than as single individuals. Nevertheless these results cannot be generalized, since sometimes through storage in certain individuals (the above-mentioned fat ants during overwintering phase or "honey-pot" ants), the situation may be quite reversed. Tracer experiments carried out with replete workers of Proformica nasuta Nyl. in our laboratory [37] seen to indicate this.

ACKNOWLEDGEMENTS

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DISCUSSION

J. LECOMTE: Although your studies on the important question of transmission of food in the form of labelled caterpillars have been confined to the laboratory, do you consider that such exchanges can be as intense as the exchange of sugared foods?

W. KLOFT: Your question is an important one, and we did take this problem into consideration. As I stated, the laboratory experiments with labelled caterpillars were positive throughout, and ought to be repeated under outdoor conditions. These caterpillars were given in the form of living prey. The specific activities of the caterpillars were not very high, but we known from our experiments that the genus Formica prefers haemolymph when feeding on insects, and takes up the cell complexes suspended therein particularly from the fat-body, and of course other tissues also. This more or less liquid precipitate can then be directly distributed in the same way by regurgitation.

D.A. CROSSLEY: I would like to ask Dr. Kloft what percentage of the P^{32} in the body would be excreted through the salivary gland and consequent passage through the gut.

W. KLOFT: We carefully measured these percentages. I do not know the exact figures but they will be published by our colleague, H. Naarmann. The total amount excreted in this way in the course of one day is about 1-5% of the total body activity remaining after there is no more primary activity in the crop. It is important to bear in mind that this only occurs with individuals during a certain phase of life, and especially with over-wintered young workers from the previous year. Workers which are born this year can do this next year, in April to May. During that time the young workers which have over-wintered feed the young queen-larvae with glandular secretions and in this way substances coming from the fat-body are used for rearing young sexual forms, especially young queens.

D.A. CROSSLEY: May I ask a further question? If you now consider the concentration of radioisotopes in various steps in the food chain, would it not be possible to measure the flow of energy through an ant nest, that is the amount of transfer of stored energy from one phase to another? You should be able, should you not, through this type of distribution, to calculate how much trophallaxis occurs, in theory at least?

W. KLOFT: We have not yet made this calculation, though it should be possible, using your special techniques for summation of the ingested activity of one queen larva, for example.

Mme. S. FUZEAU-BRAESCH: May I ask Dr. Kloft something about his experiment on measuring activity in isolated animals and grouped animals? He finds that the biological half-life of isolated animals is less than that of animals in groups, and mentions a group-effect phenomenon in that con-

nection. I would like to ask him whether he thinks that it is a real group-effect – that is, an effect which goes through sensorial and nervous stimuli and acts on the whole physiology of the animal – or simply a result of the grouping of several individuals leading to recontamination, trophallactic or otherwise.

W. KLOFT: Naturally I am aware that the group-effect must to a large extent be conditioned by sensorial and nervous stimuli. We regard it as a complex phenomenon in which the mutual exchange of food and other substances plays a contributory part, as one single factor. Through the continuous exchange of crop-content with or without glandular secretions by licking and so on, essential substances as well as pure calorie providers, (i.e. pure energy foods) are distributed evenly throughout the group; not every individual has these or is always in a physiological condition to produce them. Radioactive phosphorous was excreted through the cuticula of queens and the excreted P^{32} was licked up by the workers and distributed among each other. We think this food transfer between individuals within a group is a main cause of their longer expectation of life.

As you know, if we isolate one single individual, a termite, an ant or a honey-bee worker, and give her all the energy substances she needs, she cannot live as long as the same individuals with the same food supply in a group. These things are well described by Grassé and his group in Paris. Through repeated exchanges of such food substances and also glandular excretions, all individuals have about the same equilibrium in foodstuffs and other substances. I know they need the stimuli, including all the olfactory and other nervous stimuli, but I think the longer biological half-life is undoubtedly related to the so-called group-effect.

G. COURTOIS: I should like to add that by using Ir^{192} we have also found this increase in the biological half-life in grasshoppers, depending on whether they are kept isolated or in groups.

D.A. CROSSLEY: Dr. Frank Gaully reports a similar increase for millipedes. He can produce the effect with an individual animal by preventing it from eating its own faeces. The particular millipede with which he is working has a strong tendency to eat a good number of its own droppings. If the animal is prevented from doing this, its measured biological half-life becomes much shorter.

Mme. S. FUZEAU-BRAESCH: Could part of the difference in the biological half-life be due to immediate recontamination between individuals, that is to a phenomenon similar to the one mentioned in the millipede?

W. KLOFT: Undoubtedly, we also have external contamination because individuals lick each other externally. We cannot prevent that. I take the biological half-life as a total effect for the whole group. These external excreted substances are always taken up from one individual to the other, and this type of external contamination is undoubtedly included, but using gamma-emitting tracers and measuring with scintillation counters, we had no problems as regards absorption effects. In other cases, as I reported at the Bombay Symposium*, we get higher rates of radioactivity, after excretions, on the cuticula. At the beginning of the experiment, using gamma-emitters, we

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* Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962), 163.

had no such problems, but I agree with you that we do have some external contamination. The substance, however, is not lost to the community. Individuals take it from one another.

Mme. S. FUZEAU-BRAESCH: I believe I could suggest a useful experiment. This prolonged sensorial effect, which is a real group-effect, concerns a whole group of insects.

To eliminate contamination I suggest that isolated animals should be measured. Some of them could be isolated from a long time, and the others kept in groups before the experiment. Since the group-effect is an effect which is prolonged, any difference in the half-life occurring will be due solely to a "group-effect".

W. KLOFT: Mutual contamination with the radioactive group is of course unavoidable. In fact, the technique is based directly on it. The animals lick each other constantly and – as we have been able to show by the tracer method – they distribute, by regurgitation-feeding, substances excreted by the cuticula. Thus in actual fact a substance already excreted returns again to the intestinal tract of other individuals or, by means of regurgitation, even enters into the metabolism of the original producer.

Nevertheless I agree with you that further differentiation should be made in the experiments, in order to solve these complex problems.

J. HALBERSTADT: In connection with biological half-life problems, and in order to get more exact data, would it not be helpful to run parallel experiments using two isotopes of the same element, P^{32} and P^{33}, for instance, or I^{131} and I^{132}? Would that not give you much more exact information?

W. KLOFT: We have not yet done the experiment with two isotopes of the same element, but our field experiments on ants were done partly with two different tracers, P^{32} and I^{131}, and we had different half-lives for these two, because P^{32} is very well assimilated in the body while I^{131} is less well assimilated. However, I will bear your suggestion in mind.

USE OF RADIOACTIVE TRACERS IN THE STUDY OF INSECT-PLANT RELATIONSHIPS

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Abstract — Résumé — Аннотация — Resumen

USE OF RADIOACTIVE TRACERS IN THE STUDY OF INSECT-PLANT RELATIONSHIPS. In early uses of radioactive tracers in ecological investigations of pests insect, dispersion and migration phenomena were studied with radioisotopes as markers for individual insects. A more recent development is the use of radioisotopes to evaluate insect-plant and predator-prey associations through estimates of food utilization. Biological elimination of radioisotopes, a nuisance in dispersion studies, is being utilized as a measure of feeding rates. In both field and laboratory experimentation, insects are allowed to reach steady-state concentrations of radioisotopes through feeding. Then the rate of intake is equal to the rate of elimination of the radioisotope: (rate of ingestion) = (steady-state amount) × (fractional rate of loss). Measurements of elimination rates (biological half-lives) permit the steady-state concentrations to be translated into intake rate functions.

Food consumption studies have been performed for single-insect-species populations and for multiple-species populations. In a single-species investigation, radiocaesium in a tagged field site was used to estimate the consumption of willow leaves by populations of the beetle Chrysomela knabi. Direct measurements of food consumption made in the laboratory showed good agreement with field estimates of feeding rates based on the radioisotope techniques. Biological half-lives differed for the larval stages and these had to be considered separately. Radioisotopes provided a means of separating overwintering adults from newly emerged ones, through the more rapid elimination of caesium from overwintering adults.

In multiple-species work, the relationship between size of insect and elimination rate was used to derive an average biological half-life for radiocaesium elimination from herbivorous insects in a field site tagged with caesium-137. This average rate, used in conjunction with data on plant and insect biomasses and concentrations of radiocaesium, permitted an evaluation of plant consumption by an entire insect community. Similarly, the utilization of insects as food by predaceous arthropods was estimated from steady-state concentrations of radiocaesium in predators and prey, biomasses, and an average elimination rate.

EMPLOI DES RADIOINDICATEURS DANS L'ÉTUDE DES RELATIONS INSECTE-PLANTE. Dans les premiers travaux écologiques sur les insectes nuisibles effectués à l'aide de radioindicateurs, les chercheurs ont étudié les phénomènes de dispersion et de migration en utilisant les radioisotopes pour le marquage d'individus. Plus récemment, ils s'en sont servis pour étudier les relations insecte-plante et prédateur-proie, en procédant à des évaluations de l'utilisation des aliments. L'élimination biologique des radioisotopes, qui constitue un inconvénient dans les études sur la dispersion, est utilisée dans ce cas comme mesure des taux d'alimentation. Dans les expériences en plein champ ou en laboratoire, on attend que la concentration des radioisotopes ingérés par les insectes parvienne à l'équilibre. Le taux d'absorption des radiosotopes est alors égal à leur taux d'élimination: (taux d'ingestion) =(quantité à l'équilibre)×(taux de pertes fractionnaire). Les mesures des taux d'élimination (période biologique) permettent de traduire les concentrations à l'équilibre en fonctions exprimant les taux d'ingestion.

Les auteurs ont procédé à des études sur la consommation d'aliments dans des populations composées d'une seule espèce d'insectes et dans des populations composées de plusieurs espèces. Dans une étude en plein champ sur une seule espèce, ils ont procédé au marquage par le radiocésium afin d'évaluer la consommation de feuilles de saule par des populations de chrysomèles (Chrysomela knabi). Les mesures directes de la consommation d'aliments faites en laboratoire ont bien corcordé avec les évaluations des taux d'alimentation faites en plein champ au moyen des méthodes radioisotopiques. Les périodes biologiques ont été différentes pour les stades larvaires, qu'il a fallu étudier séparément. Les radioisotopes ont permis de distinguer les adultes ayant survécu à l'hiver de ceux qui venaient d'apparaître; en effet, le césium est éliminé plus rapidement par les premiers.

Dans les études portant sur plusieurs espèces, on s'est servi du rapport entre la taille de l'insecte et le taux d'élimination pour établir une période biologique moyenne d'élimination du radiocésium chez les insectes herbivores dans un champ marqué au césium-137. En rapprochant cette moyenne des données relatives aux ensembles biologiques de végétaux et d'insectes et aux concentrations du radiocésium, il a été possible d'évaluer la consommation de végétaux pour toute une population d'insectes. De même, à partir des concentrations à l'équilibre du radiocésium dans les prédateurs et leurs proies, ainsi que dans les ensembles biologiques, et du taux moyen d'élimination, on a évalué l'importance des insectes dans la nourriture des arthropodes prédateurs

ИСПОЛЬЗОВАНИЕ РАДИОАКТИВНЫХ ИНДИКАТОРОВ ДЛЯ ИЗУЧЕНИЯ ВЗАИМНОЙ СВЯЗИ МЕЖДУ НАСЕКОМЫМИ И РАСТЕНИЯМИ. При более раннем использовании радиоактивных индикаторов в экологических исследованиях насекомых-вредителей их рассеяния и миграции изучались при помощи радиоизотопов, которыми маркировались отдельные насекомые. В последнее время получило распространение использование радиоизотопов для изучения биологической ассоциации насекомых и растений, а также хищных насекомых и их добычи, исходя из определения потребления ими пищи. Биологическое выделение радиоизотопов, которое мешает изучению рассеяния насекомых, используется в настоящее время для измерения количества потребления пищи. Как в полевых исследованиях, так и в лабораторных экспериментах насекомым дается возможность достичь в процессе питания состояния устойчивой концентрации радиоизотопов. В этих условиях темп поглощения данного радиоизотопа равен темпу его выделения: (темп поглощения) = (величине устойчивого состояния) × (скорость потери). Измерения темпов выделения (биологического полураспада) позволяют переводить состояния устойчивой концентрации в функции темпов поглощения.

Изучение потребления пищи было проведено как для популяций насекомых одного только вида, так и для популяций смешанного типа. При изучении насекомых одного вида на меченом поле использовался радиоактивный цезий для определения потребления ивовых листьев популяциями жука Chrysomela knabi. Проведенные в лаборатории непосредственные измерения потребления пищи показали хорошее совпадение с результатами изучения темпов питания, полученными на опытном поле на основании радиоизотопного метода. Однако для личинок биологический период полураспада оказался иным, так что эти результаты должны рассматриваться раздельно. С помощью радиоизотопов удалось разделить перезимовавших взрослых особей от вновь появившихся на свет, благодаря более быстрому выделению цезия перезимовавшими взрослыми особями. При работе со смешанными популяциями было использовано соотношение между размером насекомого и темпами выделения радиоизотопов для определения среднего биологического периода полураспада при выделении радиоактивного цезия травоядными насекомыми, меченными цезием-137. Эти средние темпы совместно с данными о биомассах растений и насекомых, а также о концентрациях радиоактивного цезия позволили определить размеры потребления растений всей популяцией насекомых. Аналогичным образом, количество насекомых, съедаемых хищными членистоногими, было определено на основании состояния устойчивой концентрации радиоактивного цезия у хищников и у их добычи, их биомасс и средних темпов выделения.

EMPLEO DE MARCADORES RADIACTIVOS PARA ESTUDIAR LAS RELACIONES INSECTO-PLANTA. Las primeras aplicaciones de los indicadores radiactivos en los estudios ecológicos sobre las plagas de insectos y sobre los fenómenos de dispersión y migración insectiles consistieron en marcar con radioisótopos a algunos insectos. Más recientemente se han llegado a utilizar los radioisótopos para estudiar las relaciones insecto-planta y depredador-víctima a través del consumo de sustancias nutritivas. La eliminación biológica de los radioisótopos, elemento perturbador en los estudios sobre la dispersión, sirve para medir los índices de alimentación. Tanto en las campañas experimentales como en los estudios de laboratorio se logra una concentración estacionaria de radioisótopos en los insectos por ingestión. El índice de ingestión de radioisótopos es entonces igual al de eliminación; la fórmula aplicada es la siguiente (índice de ingestión) = (cantidad estacionaria) × (índice fraccional de pérdida). La medida del índice de eliminación (período biológico) permite determinar el índice de ingestión a partir de los datos relativos a la concentración estacionaria.

Se han estudiado el consumo de alimentos en poblaciones insectiles formadas por una sola especie y formadas por varias especies. En el estudio de una de las primeras, se utilizó el radiocesio aplicándolo en una zona determinada para calcular el consumo de hojas de sauce por poblaciones del escarabajo Chrysomela knabi. Las medidas directas del consumo de alimentos, realizadas en laboratorio, dieron resultados que coincidían con los obtenidos en las campañas experimentales emprendidas para calcular los índices de alimentación mediante el uso de radioisótopos. En la fase larval, el período biológico de los insectos es diferente, por lo que hubo de ser objeto de un estudio aparte. Los radioisótopos permitieron distinguir a los adultos que habían pasado la hibernación de los adultos nuevos, porque la eliminación del cesio en los primeros era más rápida.

En los estudios sobre poblaciones formadas por varias especies, la relación entre el tamaño del insecto y el índice de eliminación sirvió para calcular el período medio biológico para la eliminación del radiocesio por insectos harbívoros en una zona tratada con ^{137}Ce. Este período medio y los datos sobre las masas biológicas vegetales e insectiles y sobre las concentraciones de radiocesio han permitido calcular el consumo de vegetales de una población entera de insectos. Del mismo modo, se ha podido calcular el consumo de insectos de los artrópodos depredadores basándose en las concentraciones estacionarias de radiocesio en los depredadores y en las víctimas, las masas biológicas y el índice medio de eliminación.

INTRODUCTION

In modern insect-control practices, an integration of chemical, biological, cultural and other manipulative procedures is increasingly visualized as the ideal means for minimizing the depredations of pest insects and mites. From the purely biological aspects, autecological work on pest insects has always been considered meritorious and ecological phenomena were prominent in the attempts at "biological control" during the past few decades. Recent concepts, however, transcend the older ideas of biological control in that pest insects are regarded as members of more extensive ecological systems. For example, SMITH [1] has listed three principles for an integrated control concept which may be restated briefly as follows: (1) Crops, pest insects, and other biota and environment should be treated as a functional unit — the ecosystem; (2) Control measures should aim to keep pests below economic levels rather than attempt complete eradication; and (3) Disruption of other parts of the ecosystem must be considered in the evaluation of control procedures.

Implicit in such concepts is a consideration of insect-crop relationships as part of a unit system and the evaluation of control measures as a shifting of balances within the system. Such an integrated control procedure is not always necessary and may even be undesirable in some instances. The evaluation of even a very effective chemical control procedure must be made on more than destruction of pest insects, however, and such evaluation falls within the realm of ecology.

Radioisotope techniques provide an effective means of studying insect-plant relationships within an ecosystem context, and the full value of these techniques evidently is yet to be realized. Early uses of radioisotopes in economic entomology involved studies of dispersal and migration phenomena [2]. The unique value of radioactive materials as markers for individual insects has become a universally appreciated technique [3]. In mass tagging experiments, the loss of ingested radioisotope due to biological elimination was noted and deemed to be of nuisance value only. DAVIS and FOSTER [4] seem to be the first ecologists to suggest that biological elimination of radioisotopes be used to measure feeding rates, although this concept is inherent in numerous physiological studies [5]. The new doctrine of health physics also has contributed functional models and terminology. Essentially these methods use elimination rates to estimate accumulation rates, so that the concentration of a radioisotope in an insect (for example) can be used to infer rates of plant consumption or prey consumption by predaceous insects. The elimination rate technique offers a widely applicable and uncomplicated means for trophic analysis.

ELIMINATION RATES AND BIOACCUMULATION

The amount of a radioisotope which is retained in an insect following ingestion of a single dose decreases exponentially, because of biological elimination. Fig. 1 shows the retention of caesium-137 by a female grasshopper (Melanoplus differentialis); the exponential decrease produces a straight line in a semilogarithmic graph. Expressions of such rates of elimination are usually made in terms of the biological half-life. This is defined as the time required for the amount of a radioactive element in the body to decrease to one-half its initial value as the result of biological processes. The data in Fig. 1 suggest a biological half-life of approximately 2.5 d. Since these data were not corrected for radioactive decay, the result is technically an effective half-life; however, the physical half-life of Cs^{137} is so long in comparison with the observed half-life (25 yr as against 2.5 d) that radioactive decay can be ignored. In the general case these three rates are related as follows:

$$T_{eff} = \frac{T_b\, T_r}{T_b + T_r} \tag{1}$$

where T_{eff}, T_b, and T_r are the effective, biological, and radioactive half-lives respectively.

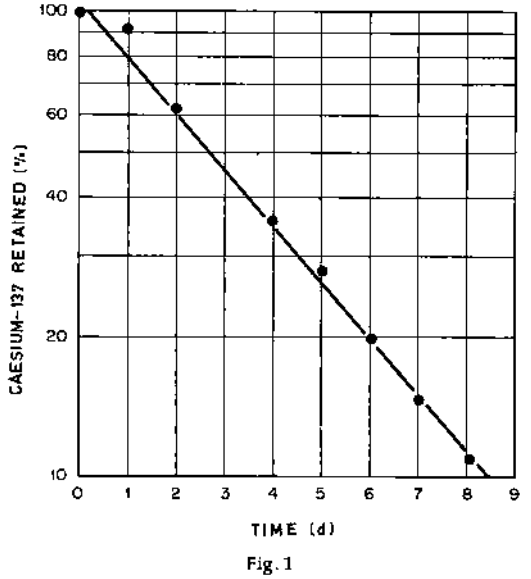

Fig. 1

Retention of Cs^{137} by a single grasshopper, Melanoplus differentialis.

By analogy with radioactive decay, the loss of radioactivity from the insect's body can be described from the relationship:

$$A_t = A_0\, e^{-ki} \tag{2}$$

where A_t is the radioactivity at any time t, A_0 is the initial radioactivity of the insect at time t = 0, and k is an elimination constant equal to $0.693/T_b$ (or $0.693/T_{eff}$ in the general case). For the case illustrated in Fig. 1, k is 0.693/2.5 d, or 0.277 d^{-1}.

The amount of radioisotope eliminated during any given day, in this example, is equal to the radioisotope content of the insect times the elimination constant k. Thus when an insect feeds continually on radioactive plants the daily rate of elimination will increase until daily intake is balanced by daily elimination. At that time the concentration of radioisotope in the insect may be said to have reached a steady state. The relation between intake and elimination in a steady state is:

$$r = kA_q \tag{3}$$

where r is the rate of feeding (μc/d), k is the elimination constant (d^{-1}), and A_q is the whole-body radioactivity (μc) in steady-state equilibrium. Thus, if the steady-state radioactivity of the insects is measured and the biological half-life for the particular radioisotope is known, the rate of feeding r can be calculated directly. If the concentration of radioisotope in the food of the insects is known, then r can be expressed as the amount of food consumed per day. If only a fraction of the radioisotope ingested is assimilated, a correction must be applied. The right-hand side of Eq. (3) may be divided by the fraction assimilated, or the elimination of unassimilated material may be treated as an additional exponential factor. No such correction is necessary for the results discussed in this report since they are based on radiocaesium, which is almost completely assimilated [6].

The approach to the steady-state equilibrium concentration is asymptotic; the time required to reach 50% of the equilibrium value is equal to one biological half-life. Practically, equilibrium is considered to be attained after a time equivalent to five biological half-lives. This would be 97% (31/32) of the final value.

APPLICATION TO FIELD POPULATIONS

Two types of field application of these radioactive tracer methods will be discussed, the single-species population and the multiple-species population, since each presents its own set of requirements.

Single-species population

Feeding rates were estimated for the third-instar larval stage of Chrysomela knabi Brown, feeding upon willow leaves. This beetle species overwinters in the adult stage. Eggs are laid on young willow leaves in early May, in the East Tennessee area. A single generation of beetles develops each year. All three larval stages as well as adults feed exclusively on willow leaves. Development is completed usually by mid-June. The life history of this beetle species has been described by BROWN [7].

Laboratory studies showed that biological half-lives for radiocaesium in this insect increased with age and size during development. Elimination

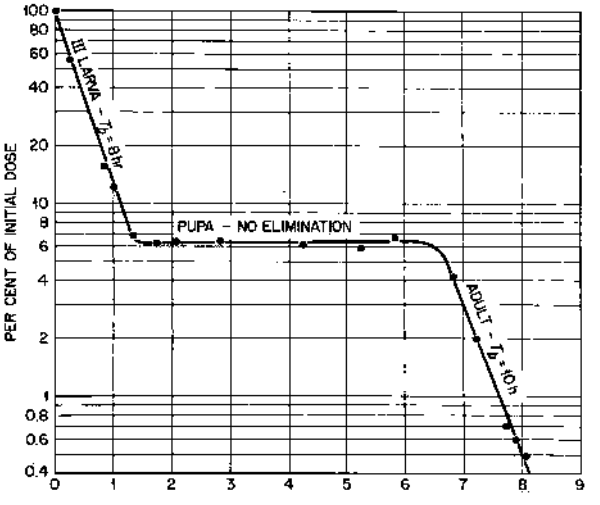

Fig. 2

Retention of Cs^{134} by an individual beetle (Chrysomela knabi)
Third-instar larva, pupa, and adult stages are shown.

of Cs^{134} occurred at rates corresponding to a 7-h biological half-life for first-instar larvae (mean of 4 individuals), an 8-h T_b for second-instar larvae (10 individuals), an 8-h T_b for third-instar larvae (14 individuals), and a 10-h T_b for newly-emerged adults. Fig. 2 illustrates Cs^{134} retention by one individual. Loss during the third instar, no elimination during the pupal stage, and further loss by the newly emerged adult are shown. These short biological half-lives suggest that a steady-state concentration would be reached in less than two days of feeding. However, the average duration of the larval stages also is short, and only during the third instar (5-d average duration) and the adult stage might a steady-state equilibration be expected.

Beetles containing Cs^{137} were obtained from willows growing on the White Oak Lake bed. White Oak Lake was formerly a part of the Oak Ridge National Laboratory's low-level waste disposal system. The lake was drained in 1955, and the plants and animals which invaded the lake basin accumulated various radioisotopes from the sediments [8]. Cs^{137} accumulated by willows (Salix nigra) growing in these sediments is transferred to insects feeding upon these plants. The consumption of leaves was calculated for third-instar larvae, which were more readily collected than were adults. The Cs^{137} content of a sample of 122 third-instar Chrysomela knabi larvae was 72.9 ± 14.6 pc/g dry wt. This value represents the steady-state equilibrium concentration. The elimination constant k, based on a biological half-life of 8 h (= 0.333 d), is estimated as $0.693/0.333 = 2.08$ d^{-1}. By substitution in Eq. (3), $r = (2.08)(72.9) = 151 \pm 30.4$ pc/g beetle per day. The average Cs^{137} concentration in willow leaves was 37.8 ± 2.38 pc/g dry wt., on the basis of leaves from which the beetle larvae were collected. Dividing 151 pc consumed per day by 37.8 pc/g gives 4.01 ± 0.84 g plant consumed/g beetle per day. The average weight of third-instar larvae in the sample was 4 mg;

TABLE I

AVERAGE WEIGHTS OF FOOD CONSUMED BY THIRD-INSTAR LARVAE OF CHRYSOMELA KNABI

Values are means for 12 measurements.

Age of larvae (days in third instar)	Mean weight of beetles (mg dry wt.)	Mean weight of food eaten (mg dry wt.)
1	1.9	6.3
2	2.4	10.7
3	4.6	15.0
4	7.4	15.4
5	9.0	5.3

the consumption of willow leaves thus would be estimated at 16.0 ± 3.4 mg dry wt. per beetle larva per day.

Table I shows the results of direct laboratory measurements of food consumption (willow leaves) by third-instar larvae. This instar is a period of rapid growth; weights of larvae increased from a mean of 1.9 mg to 9.0 mg in the 5-d period. Food consumption showed an increase from 6.3 to 15.4 mg/d, followed by a sharp drop to 5.3 mg/d on the day preceding pupation. The average weight of the third-instar larvae collected from willows on White Oak Lake bed was 4 mg; in Table I this corresponds to a daily food consumption of about 15 mg, as compared with the field estimate of 16 mg inferred by the radiocaesium technique.

These results show the accuracy which can be obtained for field data by this radioisotope technique. Biological half-lives are probably slightly different for each day of prepupation in the third larval instar, as well as between instars, but additional refinements in estimates of elimination constants would appear unnecessary. The results may be exceptionally good in this example, since the insects sampled could be related to a particular leaf on which they were feeding and thus sampling error was reduced for estimates of leaf concentrations of Cs^{137}. Had the sampling been based on adults, the motility of that stage might have reduced the accuracy in field estimates. An anomaly in the biological half-lives of Cs^{137} in adult beetles was also noted: the average T_b was 10 h for new adults but only 7.5 h for year-old ones. This difference is probably a reflection of the metabolic status of the beetles. New adults were accumulating materials to prepare for hibernation, whereas year-old ones were mating, seeking oviposition sites and laying eggs. The radioisotope equilibration technique would have estimated different feeding rates for the two groups of adults, other things being constant. Laboratory information was not sufficient to show whether feeding rates really differed for new and year-old adults.

Multiple-species populations

Areas with natural vegetation may easily support several hundreds of different species of insects, and ecological measurements become infinitely more complicated in such communities. Direct measurement of food consumption becomes difficult if not impossible. Indirect estimates must be made if generalizations are to be applied to the entire insect community, and approximate techiques become particularly attractive. The radioisotope equilibration method of Eq. (3) can be applied to multiple-species populations as well as to single-species populations. The rate of plant consumption by the insect community living on the above-mentioned White Oak Lake bed has been estimated through this radioisotope technique by CROSSLEY and HOWDEN [6], and the theoretical validity has been discussed by CROSSLEY [9]. Additional samples of the flora and fauna of White Oak Lake bed, taken during the summer of 1961, form the basic data for the present discussion. The results are similar to the estimates reported previously [6, 9] but some differences noted in the discussions can be ascribed to unusually cool weather in the summer of 1961.

An appropriate value must be selected for the elimination constant k of Eq. (3). Ideally this value would be some sort of weighted average for all of the insect species feeding on vegetation on the White Oak Lake bed. Clearly it is impractical to measure radioisotope elimination rates for each

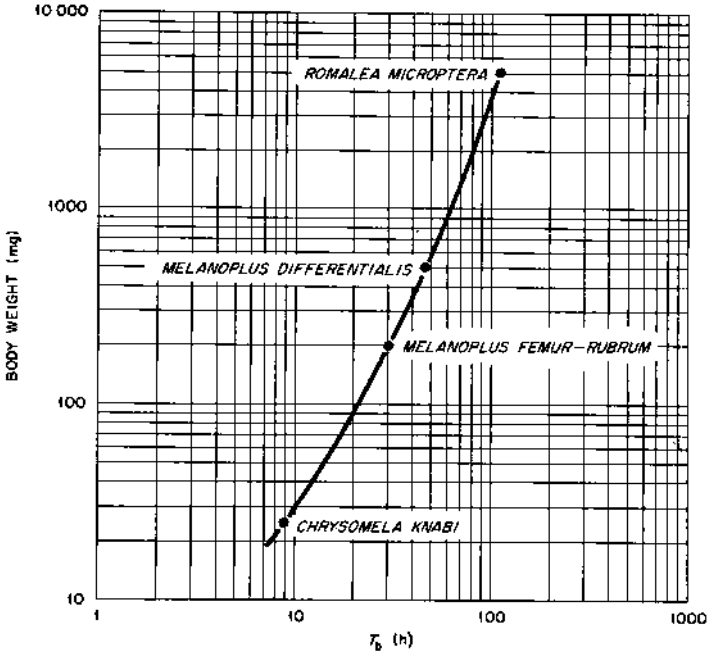

Fig. 3

Relation of insect weight to biological half-life of caesium.
(After CROSSLEY [9])

of the several hundreds of species. For caesium, however, the biological half-life has been found to be related to the size of the insect [9]. Fig. 3 shows the biological half-life of caesium in insect species plotted as a function of fresh weight (mg) of the insects. Biological half-lives of caesium in mammal species, as reported by LANGHAM and ANDERSON [10], fall close to a projection of the line in Fig. 3. Weights of insect species can thus be made the basis for selection of an average k for the entire community. For the present discussion we shall use a weighted average weight per insect collected in the 1961 samplings, which was approximately 20 mg fresh wt. The line in Fig. 3 suggests a biological half-life for caesium of about 7.5 h for this weight. This is 0.313 d, and the average elimination constant k is estimated as 2.21 d^{-1}. This is larger than the elimination constant of 0.98 d^{-1} estimated for the 1958 samplings [9] but evidently there was a real difference between the average size of insect in 1958 and that in 1961.

TABLE II

CONCENTRATIONS OF CAESIUM-137 AND BIOMASSES FOR SOIL AND TROPHIC LEVELS OF THE PLANT-INSECT FOOD CHAIN ON WHITE OAK LAKE BED, SUMMER 1961

Mean ± standard error; number of samples in parentheses.

	Caesium-137 concentration (pc/g dry wt.)	Biomass (g/m^2)
Soil	7300 [a]	-- [b]
Plants (leaves)	160 ± 32 (5)	317 ± 42.4 (4)
Herbivorous insects	78 ± 4.8 (5)	0.111 ± 0.0192 (8)
Predaceous insects	73 (8 samples lumped)	0.0117 ± 0.0015 (8)

[a] Soil value based on grand mean from core samples.

[b] No samples taken.

The basic data obtained in the 1961 samplings are given in Table II. Concentrations of Cs^{137} in plants (leaves) and in herbivorous and predaceous insects are essentially the same as those reported for 1958 [6]. Biomasses, however, are lower, evidently as a result of cooler weather in the summer of 1961. Plant biomass dropped from about 600 to about 300 g/m^2; the average herbivorous insect biomass dropped from approximately 250 to approximately 100 mg/m^2. Final biomass samples at the end of the summer approached 250 mg/m^2, however.

Using the data in Table II, one can estimate the average rate of feeding r for the herbivorous insects as follows: (2.21)(78) = 172 pc/g insect per day.

Dividing by plant concentration (160 pc/g), one obtains r = 1.08 g plant/g insect per day. This is 0.120 g plant/m^2 d for a biomass of 111 mg/m^2, or 12 g/m^2 in a 100-d growing season. The proportion of the plant biomass consumed by insects would thus be 3.8%, which compares favourably with the 4% estimate reported for the 1958 data [9].

This amount of plant consumption is the amount necessary to maintain the steady-state equilibrium concentration in the herbivorous insects. A certain amount of the insect biomass is lost through mortality during the season, and is offset (or more than offset) by insect production. This production represents an additional consumption of plants by insects, for which we have no measure in this mixed insect community. However, estimates of feeding for production in various communities are usually about 25% of the amount of feeding required for maintenance [11]. If this is true for the insect community on White Oak Lake bed vegetation, the total average daily consumption of plants would be about 0.150 g, or 15 g per season, or 4.7% of the plant biomass consumed during the entire growing season.

Similar calculations can be made for predaceous insects from the data in Table II. If all of the predators are assumed to feed on herbivores, and thus secondary predation is ignored, r can be estimated as 2.06 g herbivore consumed per g predator per day, or 0.024 g herbivore consumed/m^2 d, or 2.4 g herbivore consumed/m^2 per growing season. This value is 15% of the amount of plant material consumed by the herbivorous insects, but 80% of the total insect production estimated above. These estimates of herbivore consumption by predators seem high. However, TEAL [12], in discussing the predator-prey balance in a temperate cold spring, estimated non-predatory mortality of herbivores at 19.4%, which is the converse of the 80% predatory mortality estimated here. The relative paucity of predaceous insects makes sampling more difficult for both Cs^{137} content and biomass estimates. Further sampling and analysis of the predator trophic level will be required to improve estimates of food consumption by predators.

DISCUSSIONS AND CONCLUSIONS

The field aspects of the two examples given here were handled under conditions unfavourable for radioactive tracer work. White Oak Lake bed contains a mixture of heterogeneously distributed radioisotope species, at levels of radioactivity generally lower than is desirable for tracer studies. These difficulties have been overcome in newly created tagged field sites. Insect consumption of foliage in a forest site tagged with Cs^{137} is being measured in a manner similar to the studies performed on White Oak Lake bed, except that control over distribution and concentration of caesium-137 in the forest system has greatly eased the problems of measurement. The accumulation of various radioisotopes by forest-floor arthropods also is being studied through steady-state concentrations and biological half-lives. In this case tagging has been accomplished by placing tagged tree leaves in small fenced (1-m^2) plots. The arthropod food chain which has as its base leaf litter is being studied in this manner.

Preliminary results from these additional studies have confirmed the value of measurements of biological half-lives for the interpretation of food-chain transfer of radioisotopes. These radioisotope techniques are fulfilling their initial promise as an uncomplicated technique for specifying rates of movement of materials along food chains, and thus quantifying the relationships of herbivorous insect populations to plants and of predators to prey.

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DISCUSSION

H. HUQUE: What was your technique for measuring radioactivity in vivo?

D.A. CROSSLEY: We used three methods: well-type scintillation counting for single insects; similar instruments for groups, so that we could measure and follow individuals and groups of insects through time; and total-faeces collections from cultures through time.

W. KLOFT: Did you include ants among predatory insects in your ecological analysis? Your method is excellent for estimating the predatory effect of ant colonies, but some complex problems arise here, such as social food exchanges within colonies.

D.A. CROSSLEY: The classification of certain insects as predators or as herbivores is bound to be an arbitrary one, since some insects will of course change their habits with changes in life history. Our method, therefore, was to consider the stage of the life-history. Larval forms which are predaceous were included as predators. A herbivorous adult was counted as a herbivore, even if the larval stage was predaceous. I believe we counted ants as herbivores.

R. von BORSTEL: Did you carry out your feeding experiments at different temperatures?

D.A. CROSSLEY: Yes, and we found that elimination rates follow approximately a Q_{10} law, i.e. for doubled metabolic activity twice the elimination rate. This suggests the use of elimination rates for radioisotopes to measure metabolic rates, which I believe to be feasible with the proper combination of isotope and insect. By selecting the proper isotope, in other words, you may be able to estimate metabolism in invertebrate populations through changes in the elimination rate.

M. FRIED: If I understood correctly, you have estimated the leaf consumption per unit of body-weight of the insect, using steady-state values of Cs^{137}. You also had the necessary data for the same calculation using steady-state values of Sr^{90}. Did these calculations give you similar results, and if not, why not?

D. A. CROSSLEY: No, strontium concentrations did not yield identical results. The discrepancy is due to less complete absorption of Sr^{90} from the intestinal tract. We made the assumption of complete absorption in these studies. That is almost true for caesium, but definitely not true for strontium.

DISTRIBUTION OF AERIALLY APPLIED MALATHION-S^{35} IN A FOREST ECOSYSTEM*

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Abstract — Résumé — Аннотация — Resumen

DISTRIBUTION OF AERIALLY APPLIED MALATHION-S^{35} IN A FOREST ECOSYSTEM. The distribution of malathion (0, 0-dimethyl dithiophosphate of diethyl mercaptosuccinate) in a forested area of east-central Ohio was studied during the summer of 1962. This broad-spectrum insecticide was selected for study on the basis of its increased use in the control of many important forest insect pests in deciduous and coniferous forests of the United States. The need for greater knowledge of the ecology of a forested area, coupled with the need for a more complete understanding of the effects of an insecticide on the fauna, provided the unique possibility of studying the problems simultaneously through the utilization of an isotope-labelled insecticide. S^{35} was selected because of its low beta energy (0.167 MeV) and the adequate half-life (87.1 d). Preliminary one-tenth-acre plot studies in the summer of 1961 provided us with potential application rates in terms of total radiation and also allowed the development of sample preparation technique. A faunal survey of two 20-acre watersheds was conducted during the summer of 1961. In May of 1962, one of the watersheds was treated with an application of 2 lb technical-grade malathion per acre in a formulation of xylene, triton X-155 emulsifier and water. The malathion was synthesized with S^{35} by the Radiochemical Centre, Amersham, England. 1 c of activity was aerially applied to one of the 20-acre forested areas on 15 May and 25 May 1962. The specific activity of the synthesized malathion was 17.5 mc/mM.

The distribution of components of the aerial spray within the forest was measured. Electrically-operated air samplers provided estimates of drift off the area; helium-filled balloons bearing frosted-glass discs measured above-canopy application; glass discs suspended vertically as well as bark samples measured quantities settling out at different layers in the canopy; glass discs and spotting-enamel paper not only allowed a measure of horizontal distribution but a check of a standard spray-distribution detection device. Soil samples and monitoring of marked stakes allowed sub-surface distribution studies.

Samples of water from the intermittent streams, insects, mammals, reptiles and birds indicated the initial and subsequent distribution of the insecticide and its metabolites in the ecosystem. Population studies of the faunal system continued throughout the summers of 1961-62 and a limited amount of survey data will be collected in the summer of 1963. Preliminary results indicate that the insect populations returned to normal in about three weeks and there was no detectable effect on the densities of the vertebrate animals on the sprayed area.

DISTRIBUTION DU MALATHION-^{35}S PULVÉRISÉ PAR AVION DANS UN ENSEMBLE ÉCOLOGIQUE FORESTIER. La distribution de malathion (0, 0-diméthyl dithiophosphate de diéthyl mercaptosuccinate) dans une zone forestière du centre-est de l'Ohio a été étudiée au cours de l'été 1962. Cet insecticide à gamme d'utilisations étendue a été choisi comme sujet d'étude à cause de son emploi croissant dans la lutte contre de nombreux insectes nuisibles aux forêts de feuillus et de conifères des Etats-Unis. La concomitance du besoin d'une connaissance plus complète de l'écologie d'une zone forestière et de la nécessité d'une compréhension plus approfondie des effets d'un insecticide sur la faune a donné une occasion unique d'étudier les problèmes simultanément grâce à l'utilisation d'un insecticide marqué avec un radioisotope. On a choisi ^{35}S à cause de sa faible énergie bêta (0,167 MeV) et de sa période (87,1 j). Des études préliminaires portant sur des parcelles de quatre ares, au cours de l'été 1961, ont permis aux auteurs de fixer des doses d'application en fonction du rayonnement total et ont permis aussi la mise au point de techniques de préparation d'échantillons. Une étude

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de la faune de deux secteurs de huit hectares, dans deux bassins différents, a été faite au cours de l'été 1961. En mai 1962, un de ces secteurs a été traité à raison de 2,25 kg par hectare de malathion commercial dans une formule comprenant du xylène, du triton X-155 comme émulsifiant et de l'eau. Le malathion avait été marqué avec ^{35}S par le Radiochemical Centre d'Amersham (Royaume-Uni). On a pulvérisé par avion une quantité correspondant à une activité de 1 c dans l'un des deux secteurs forestiers de huit hectares, les 15 et 25 mai 1962. L'activité spécifique du malathion marqué était de 17,5 mc/mM.

On a mesuré la distribution des composants de la pulvérisation aérienne dans la forêt. Des échantillonneurs d'air électriques ont permis d'estimer les quantités emportées hors du secteur d'expérience; des ballons d'hélium portant des disques de verre dépoli ont permis de mesurer la quantité en suspension au-dessus de la frondaison; des disques de verre suspendus verticalement et des échantillons d'écorces ont permis de mesurer les quantités à diverses hauteurs de la frondaison; des disques de verre et du papier pour analyse à la touche ont permis, non seulement de mesurer la distribution horizontale, mais aussi de contrôler un appareil courant de détection de la distribution des produits pulvérisés. Des échantillons du sol et des pieux marqués ont permis d'étudier la distribution au-dessous de la surface.

Des échantillons d'eau provenant de cours d'eau intermittents, des insectes, des mammifères, des reptiles et des oiseaux ont indiqué la distribution initiale et ultérieure de l'insecticide et de ses métabolites dans l'ensemble écologique. Des études de la faune ont continué pendant les étés 1961 et 1962 et certaines observations seront encore faites au cours de l'été 1963. Les résultats préliminaires indiquent que les populations d'insectes sont revenues à la normale au bout d'environ trois semaines et qu'il n'y a pas eu d'effets décelables sur la densité des vertébrés dans la surface traitée.

РАСПРЕДЕЛЕНИЕ МАЛАТИОНА, МЕЧЕННОГО S^{35} И РАСПЫЛЯЕМОГО С ВОЗДУХА ДЛЯ ИЗУЧЕНИЯ ЭКОЛОГИЧЕСКОЙ СИСТЕМЫ ЛЕСНЫХ МАССИВОВ. Распределение малатиона (О,О-диметилдитиофосфат диэтилмеркаптонитарной кислоты) в лесном массиве восточной части Центрального Огайо, США, изучалось весной 1962 года. Этот инсектицид широкого действия был выбран для изучения в связи с его возрастающей ролью в борьбе с многими важными насекомыми-вредителями лиственных и хвойных лесов Соединенных Штатов. Для расширения знаний по экологии лесных массивов, а также более полного понимания влияния инсектицидов на фауну была использована своеобразная возможность изучения этих проблем одновременно посредством применения меченных изотопами инсектицидов. S^{35} была выбрана ввиду низкой энергии бета-излучения (0,167 Мэв) и достаточно короткого периода полураспада (87,1 дня). Предварительное исследование участка земли 0,1 акра летом 1961 года показало степень возможного применения в условиях общей радиации, а также возможность разработать подготовительные методы для взятия проб. Летом 1961 года был проведен обзор фауны двух бассейнов реки площадью в 20 акров каждый. В мае 1962 года один из них был обработан технически-чистым малатионом в количестве 2 фунтов на 1 акр в смеси с кошлоном, тритоном Х-155 (эмульгатор) и водой. Малатион, меченный S^{35}, был синтезирован в Великобритании в Радиохимическом центре Эмершем. Малатион общей активностью 1 кюри был распылен на лесном массиве 20 акров 15 и 20 мая 1962 года. Специфическая активность синтезированного малатиона была 17,5 милликюри на миллимоль.

Измерялось распределение компонентов при воздушном распылении в пределах лесного массива. С помощью электрически управляемых пробоотборников воздуха производилась оценка границ зоны распыления; с помощью наполненных гелием баллонов с охлаждаемыми стеклянными дисками измерялось осаждение над кроной; с помощью вертикально установленных стеклянных дисков, а также образцов коры измерялись количества осаждающегося малатиона в различных горизонтальных слоях леса; с помощью стеклянных дисков и специальной бумаги с эмалиевым вкраплением можно было измерять горизонтальное распределение и проверять работу стандартного устройства для изучения распределения при распылении. Измерение образцов почв и маркированных вех позволяло изучить подповерхностное распределение.

По образцам воды, взятым из ручьев, насекомым, млекопитающим, пресмыкающимся и птицам можно было судить о первоначальном и последующем распределении инсектицида и его продуктов обмена в экологической системе. Изучение обитателей леса проводилось в летнее время на протяжении 1961 - 1962 годов, и некоторое количество обзорных данных будет получено летом 1963 года. Предварительные результаты указывают, что количество насекомых восстановится до нормального уровня примерно через три недели и что не наблюдается заметного воздействия на количество позвоночных животных, обитающих в зоне распыления.

DISTRIBUCIÓN EN UN SISTEMA ECOLÓGICO FORESTAL DE MALATIÓN-^{35}S PULVERIZADO DESDE EL AIRE. Durante el verano de 1962 se estudió la distribución del malatión (O,O-dimetil ditiofosfato del mercaprosuccinato de dietilo) en una zona forestal de la región centro-oriental de Ohío (Estados Unidos). Se eligió este insecticida de espectro tan amplio su empleo se generaliza cada vez más para combatir muchas plagas de insectos importantes en los bosques de coníferas y de árboles de hoja caediza de los Estados Unidos. El empleo de un insecticida marcado con un isótopo radiactivo permite estudiar simultáneamente la ecología de una zona forestal y los efectos del insecticida sobre la fauna. Los autores de la memoria eligieron el ^{35}S debido a su reducida energía beta (0,167 MeV) y a que su período de semidesintegración (87,1 d) resulta conveniente. Los estudios preliminares realizados en verano de 1961 en parcelas de cuatro áreas permitieron determinar las dosis de aplicación posibles en función de la irradiación total, y establecer una técnica de preparación de las muestras. En el mismo verano se estudió la fauna en dos vertientes de ocho hectáreas. En mayo de 1962 una de ellas fue tratada con cinco libras de malatión de calidad comercial por hectárea, disuelto en xilol y emulsionado en agua con Tritón X-155. El malatión marcado con ^{35}S fue sintetizado en el Radio Chemical Centre de Amersham (Gran Bretaña). El 15 y el 25 de mayo de 1962 se aplicó desde el aire un curie de actividad a una de las vertientes de ocho hectáreas. La actividad específica del malatión era de 17,5 mc/mM.

Los autores midieron la distribución de los componentes del insecticida pulverizado sobre el bosque. Aparatos eléctricos de toma de muestras de aire permitieron calcular la parte del insecticida que pasó a las zonas colindantes; el aplicado por encima del nivel de las copas de los árboles se midió con globos de helio portadores de discos de vidrio esmerilado; las cantidades depositadas a diversos niveles del follaje se midieron con discos de vidrio colgados verticalmente y con muestras de corteza; la distribución horizontal se determinó con discos de vidrio y papel especial, que sirvieron también para ensayar un dispositivo destinado a determinar la distribución del insecticida pulverizado. Por toma de muestras de tierra y recuento de zonas delimitadas se estudió la distribución bajo la superficie del suelo.

Las muestras de agua tomadas en arroyos intermitentes y los ensayos realizados con insectos, mamíferos, reptiles y aves indicaron la distribución inicial y subsiguiente del insecticida y sus metabolitos en el sistema ecológico. Los estudios de la población animal siguieron durante los veranos de 1961 y 1962; en el verano de 1963 se reunirán todavía algunos datos. Los resultados preliminares indican que la población de insectos vuelve a la normalidad unas tres semanas después del tratamiento; no se observó efecto alguno en los animales vertebrados de la zona tratada.

INTRODUCTION

A greater understanding of the complexities of man's total environment is necessary for the maintenance and improvement of our standard of living and for our survival. One aspect of our environment, the forest, making up about one third of the vegetative cover of the land, has profound influences on man, topography, climate, and use of other natural resources.

The need for a greater ecological understanding of forested areas has become evident to biologists as man intensifies his use of such lands for wood products, watersheds, wildlife habitat, and recreation. Parallel needs for an understanding of pesticidal effects on forests have become obvious to many people following widespread use and misuse of these chemicals.

Insecticides play an integral part in the management of productive land with approximately 92 million acres treated annually in the United States, 22% of which is aerially treated. Millions of acres are sprayed world-wide for protection of trees and control of disease vectors. True multiple use of forested and agricultural areas can only be accomplished with an appreciation for the entire biotic community, including man. Our study presented an unusual opportunity to study the problems of insecticides and forest ecology simultaneously for mutual benefit.

The need for more knowledge in these complex fields of investigation is evidenced by our meeting here to discuss mutual problems and exchange ideas.

THE INSECTICIDE MALATHION

Malathion, 0, 0-dimethyl dithiophosphate of diethyl mercapto-succinate ($C_{10}H_{19}O_6PS_2$) is a broad-spectrum, general-purpose, residual organophosphate insecticide and acaricide. Extensive reviews of the use and characteristics of malathion are readily available in the literature [1, 2, 3], that of SPILLER [4] being very comprehensive. Malathion ranks second only to Parathion in world production of organo-phosphates, over 5000 tons being produced annually [5].

Since we are primarily concerned in this report with an ecological problem, a complete review of the mode of action and metabolism of malathion does not seem warranted. The insecticide is converted to the oxygen analogue, malaoxon, which reacts in vivo as an anticholinesterase [6]. One of the most important properties of the insecticide and a justifiable reason for its widespread use is its low mammalian toxicity. O'BRIEN [6] describes this metabolism or detoxification performed primarily by the liver, which makes it unavailable to bird and mammal blood-stream cholinesterases.

We selected malathion for this study because it has great potential use in forest insect control, has been extensively studied, has a low mammalian toxicity and has a short residual action. Malathion has been successfully labelled with P^{32} and S^{35}. We selected the latter isotope primarily because of its longer half-life. The Radiochemical Centre, Amersham, Bucks, England, synthesized a small quantity of the material for us in 1961 and produced 1 c of S^{35}-labelled malathion for delivery in May 1962. The radiochemical purity of the synthesized insecticide was checked by paper chromatography. The material was tested 96% pure and was assayed on 13 May 1962 at a specific activity of 17.5 mc/mM. The malathion was delivered to our project area in four ampoules containing a total of 1051 mc of activity. The radioactive insecticide was formulated at the project area with xylene solvent (30%), Triton X-155 emulsifier (8%), and water at the rate of 2 lb technical-grade malathion (62%) to each acre treated. The one-swath application on 15 May 1962 was made at the rate of 10 US gal of formulated material per acre, while the 25 May application was made at the rate of 5 gal per acre. Each application was at 2 lb per acre technical-grade malathion. The earlier one-swath application was made with a Piper J-3 aircraft and the latter on 25 May was applied with a Piper P-18 aircraft. The aerial application was under the supervision of The Ohio State University School of Aviation.

DESCRIPTION OF STUDY AREA

The two forested watersheds under study total 41.5 acres and are located in Tuscarawas County in the unglaciated eastern portion of Ohio, United

Fig. 1

An aerial photograph of the two watersheds studied (Dover, Ohio 1962): the southern (lower) watershed was treated, the northern served as a control.

States of America (Fig. 1). The gradient of the wooded slopes of the watersheds ranges from 5 to 40% and is covered by a second-growth dry mixed mesophytic hardwood forest, composed largely of yellow poplar (Liriodendron tulipifera), white oak (Quercus alba), red oak (Quercus borealis), red maple (Acer rubrum), and black oak (Quercus velutina). Timber stocking is about 70 ft^2 of basal area per acre, and approximately 6500 board feet per acre. The shrub layer consists largely of sassafras (Sassafras albidum), spice bush (Lindera benzoin), maple-leaf viburnum (Viburnum acerifolium), and the saplings of the various tree species (Fig. 2).

The study area was commercially clear cut about 1900 and, except for a stand-improvement cut made in 1948 and a few commercial trees taken from the ridges in 1949, it has remained in a natural state. Ground fires apparently burned over the watersheds during the early 1930's. The area

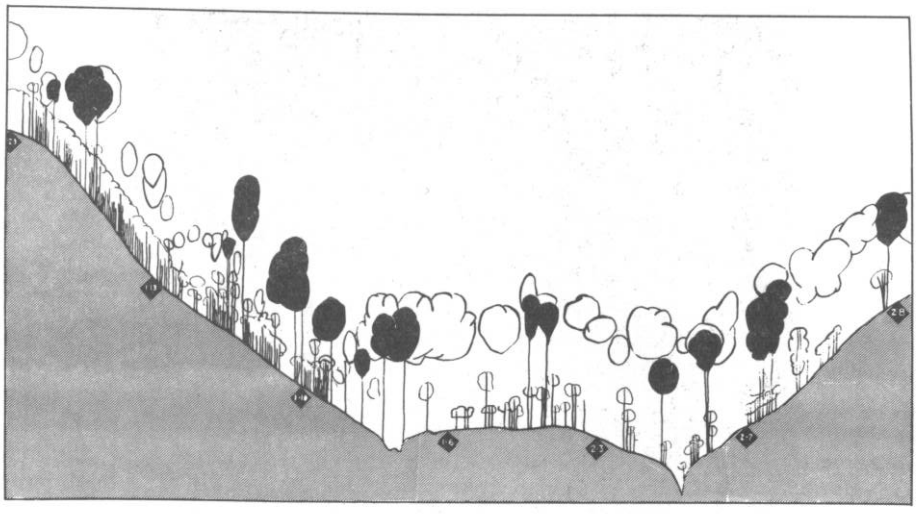

Fig. 2

A vegetative transect diagonally across both watersheds showing all woody stems 10 or more feet tall (Dover, Ohio 1962)
Trees within the transect 35 or more feet tall are shown with black canopies; canopies intercepted by the transect are shown in white.
The horizontal to vertical ratio is 4:1; there are 290 ft between numbered squares.

is currently part of a 160-acre tract leased to the United States Forest Service for watershed research purposes.

METHODS OF STUDY

Preliminary project planning began in the summer of 1960 and actual field observations were initiated in March 1961. The two watersheds, one of 23.4 acres and the other containing 18.1 acres, were surveyed and marked with a grid of 200 ft. Each grid intersection acted as a central sampling point for the various segments of the faunal system.

The problem of designing an adequate sampling scheme for the entire faunal system of the watersheds under study was recognized and the work programme was adjusted to provide the most satisfactory data within the limits of funds and manpower available. Table I lists the methods used to sample the various segments of the animal populations occuring on the study areas. Some of the methods proved successful and provided adequate information for comparing differences in populations. Major problems occurred in sampling reptiles, amphibians and the invertebrates found in the small intermittent streams. Any stream-bottom sampling method described in the limnological literature so altered the limited habitat available that these methods were not acceptable. Drift fences and traps designed to capture reptiles moving across the study units were not successful.

Sampling in 1961 and 1962 took place between April and September. Limited samples of certain species of insects will be taken during the

TABLE I

SUMMARY OF SAMPLING AND OBSERVATIONAL TECHNIQUES USED WITH THE FAUNAL SEGMENTS DURING THE STUDY
Dover, Ohio 1961-62

Soil micro-and macro-fauna	Soil CO_2 output
	Plate counts
	Berlese funnel extractions from standard-size nylon net bags
	Cryptozoan oak drop boards
Insects and invertebrates	Light trap
	Sweep net
	Earthworm extractions
	Molasses trap
	Sticky boards suspended
	Stream drift nets
	0.5-m^2 quadrats
	Stream bottom sample
	Random collecting
	Cryptozoan oak drop boards
	Tree-trunk sticky bands
Molluscs	Random collection
	Quadrat counts
Amphibians	Roofing-felt quadrats
	Random observations
	Streamside counts
	Sherman-trap counts
Reptiles	Random capture and numbering of turtles
	Snake and turtle drift-fence traps
	Random capturing and marking of snakes
	Tar-paper quadrats
Birds	Early-morning transect lines
	Breeding-bird observations
	Random observations

TABLE I (cont.)

Mammals	Large mammal live traps
	Sherman live traps
	Transect lines for mammal holes
	Mammal holes under drop boards
	Plastic tile dropping boards
	Feeding activity along hole transect lines
	Large-mammal burrow counts
Fish	Stream cages and buckets on plots

summer of 1963 in addition to S^{35} residue analyses in both vertebrates and invertebrates.

Table II presents data on the formulation and treatments applied to the watersheds in 1962.

RADIOACTIVE SAMPLE PREPARATION TECHNIQUES

All specimens collected for radioassay of S^{35} were either air-dried, placed in preservative, frozen, or brought to the laboratory in plastic bags and processed within 24 h. Information on each specimen included month, day, number of collection for each day, location, description, date received by the taxonomist, date received in the laboratory, date of the radioactive assay, and the final disposal.

Techniques described by JEFFAY et al. [7] were used with modifications to determine the level of S^{35} in each sample. The final $BaSO_4$ precipitate on a Millipore filter * in an aluminium planchet was assayed in a thin-window gas-flow counter. Analysis of the resultant data were made on the basis of statistical tests of sampling errors as described by JARRETT [8].

Spray deposition and assessment throughout the study was made on 1-in (2.54 cm) diam. circular glass discs, frosted on one side. After air-drying, these were placed on planchets and counted as described for the filtered samples.

FACTORS AFFECTING ISOTOPE DISPERSAL

There are so many factors of unknown magnitude affecting isotope dispersal that a realistic appraisal of such dispersal within an ecosystem is presently impossible. The immediate and real problems of studying dispersal of simple fall-out nuclides such as Sr^{90} or Cs^{137} are extremely

* Type HAWPO 2500

** Nuclear Chicago Model C-115

TABLE II

SUMMARY OF SPRAY FORMULATION DATA FOR TWO APPLICATIONS OF MALATHION-S^{35} Dover, Ohio 1962

Crash flight	
Date	15 May 1962
Area treated	1 spray swath (approx. 4 acres)
Rate malathion	unknown, (2 lb/acre planned)
Volume of formulation per acre	10 gal/acre
Loads	4 planned, 1 executed
Total volume of each load	58.5 gal formulation
Total volume to be applied	234 gal formulation
Malathion per load	11.7 lb
Xylene per load	1.41 lb
Triton X-155 emulsifier per load	0.38 lb
Malathion -S^{35} in load	4.98 g; 263 mc
Water per load	55.1 gal
Estimated amount applied	10-20 gal
Estimated amount entering soil at crash site	30-40 gal
Type aircraft	Piper J-3
Final flight	
Date	25 May 1962
Area treated	23.4 acres
Rate malathion	2 lb/acre
Volume of formulation per acre	5 gal
Loads	3
Total volume of each load	39 gal
Total volume to be applied	117 gal
Malathion per load	15.6 lb
Xylene per load	1.86 lb
Triton X-155 emulsifier per load	0.5 lb

TABLE II (cont.)

Malathion-S^{35} per load	4.98 g. 263 mc
	4.92 g. 260 mc
	5.02 g. 265 mc
Total malathion S^{35} applied final flight	14.92 g. 788 mc
Water per load	35.6 gal
pH	Approx. 8
Amount malathion S^{35} per acre	0.638 g
Activity per 10-µl formulation 13 May 1962	3220 counts/min
Activity per 10-µl formulation 26 Feb. 1963	323 ± 6.44 counts/min
Type aircraft	Piper P-18

complex. Work with a tagged insecticide whose complex natural breakdown products are yet unnamed, whose in vivo characteristics are yet incompletely understood, and whose complete in vivo metabolism is unknown, is many times more complex. SCHMIDT and WEIDHASS [9] comment that radioactivity in insects may represent the original insecticide, its metabolites, or both. At any specific time an equilibrium may be established between the amount taken up and that excreted. These vary with the insecticide and the insect.

AUERBACH [10] presents some factors needed to evaluate the effects of a radionuclide which are all related to dispersal and measurement of dispersal. The factors are: quantity of nuclide, uptake, mode of entrance into the body, body retention, critical organ retention, biological half-life, radioactive half-life, and the energy and type of the radiation.

Many of these factors are now being studied in our project. We shall report only on those studies now largely completed, namely, quantity and distribution of the insecticide and insecticide effects.

ISOTOPIC MEASUREMENT OF SPRAY DISTRIBUTION

LADD et al. [11] and JENKINS and DAVIS [12] used isotopes for studying the dissemination of airplane sprays. Isotopes have the advantages over dyes of greater accuracy, more rapid assessment, fewer personnel required and ability to measure depositions on vegetation and uneven surfaces without panels or other sampling devices. Additional advantages include: independence of light, temperature, pressure and chemical state of the nuclide; no solubility problems as with dyes; dilution phenomena of isotopes are well known; samples are easy to prepare; and the method is applicable

with volatile chemicals. LADD et al. [11] found that dye and isotope methods agreed within 20%, the isotope tracer giving higher values. They state that the practical limiting sensitivity of the tracer method is more than 100 times that of the dye procedure.

The disadvantages are greater expense, half-life decay and the need for special counting equipment and safety precautions. We agree with LADD et al. [11] that a nuclide with a half-life of 1 to 30 d is most desirable for simple dispersal studies. Where factors of molecular-tagging or other project objectives must be considered these limits do not apply. If the half-life is too short, the isotope deteriorates too rapidly for suitable field work. If the half-life is too long, the area and the equipment may be contaminated too long for desirable subsequent tests.

LADD et al. [11] used 60 mc of Mn^{54} naphthenate in a DDT formulation to test spray dispersal. They used two 6 × 36-in blotter panels and one 11-in plate every 30 ft along two 375-ft strips. They could detect quantities to 0.001 lb/acre of DDT by rolling blotters around the G-M tube and counting the activity. JENKINS and DAVIS [12] used Au^{198}.

Many factors modify the dispersal of aerial spray. From the nozzle, where droplet size and production are relatively unknown for the conditions of flight, to the ground, where all factors have had their effect, knowledge of spray-particle size and behaviour is needed.

Like most insecticide distribution studies, this one was concerned with final spray size and amount of application per unit area. The observed particle size is a droplet that has undergone the action of all the environmental factors and has escaped interception except by the sampling device, which also modifies its size expression. DAVIS and ELLIOTT [13] state that: "The indicated deposit may be expected to show some reduction due to evaporation. The amount of reduction will be somewhat proportional to the vapor pressure of the insecticide at the ambient temperature and to the time required for the spray to settle, and inversely proportional to the square of the drop size."

Most aerial applications have shown a loss in insecticides before they reach the ground or sampling devices. SAILER and ROZEN [14] observed that malathion applied by biplane at 2 lb/acre was deposited at 0.063 and 0.034 lb/acre; at a rate of 1 lb/acre it was deposited at 0.040 and 0.054 lb/acre. These measurements were from oil-sensitive spray-assessment cards placed at 66-ft intervals along diagonal sampling lines in each plot under study. COPE [15] reported that an application of 1 lb/acre of malathion on alfalfa near Jackson, Wyoming, resulted in 0.53 and 0.73 ppm reaching the ground. He noted that up to 0.04 ppm was in nearby water, although direct spraying of waterways had been avoided. DAVIS et al. [16] list 18 comparisons of lb/acre applied and lb/acre deposited. The mean ratio was 1:0.2.

The deviation of spray from the flight path has been noted by several authors [17,18]. CARLETON et al. [18], for example, state: "Research today indicates that spray discharged along the lateral axis of an airplane will follow the air currents set in motion by its passage through the air, until these forces will no longer support the weight of the spray droplet. These principal airstreams, which affect spray distribution, are generated by an airplane in flight. These are the right and left wingtip vortices and

the propeller vortex. These vortices, plus the effect of gravity, determine the path of the spray discharge and the resultant deposit pattern."

They note that sprays are rotated to the side by the plane propeller. The results of a spray-plane crash on 15 May 1962 in the study area after one swath are shown in Fig. 3. The wide variability of the deposition pattern can only generally be explained. Wind velocity at ground level was less than 2 mile/h. The above mentioned "aerodynamic forces" offer the only explanations. The high concentration "island" to the west is most difficult to explain. It indicates variability and, practically, points up the unlikelihood of avoiding waterways by paralleling them "a reasonable" distance away.

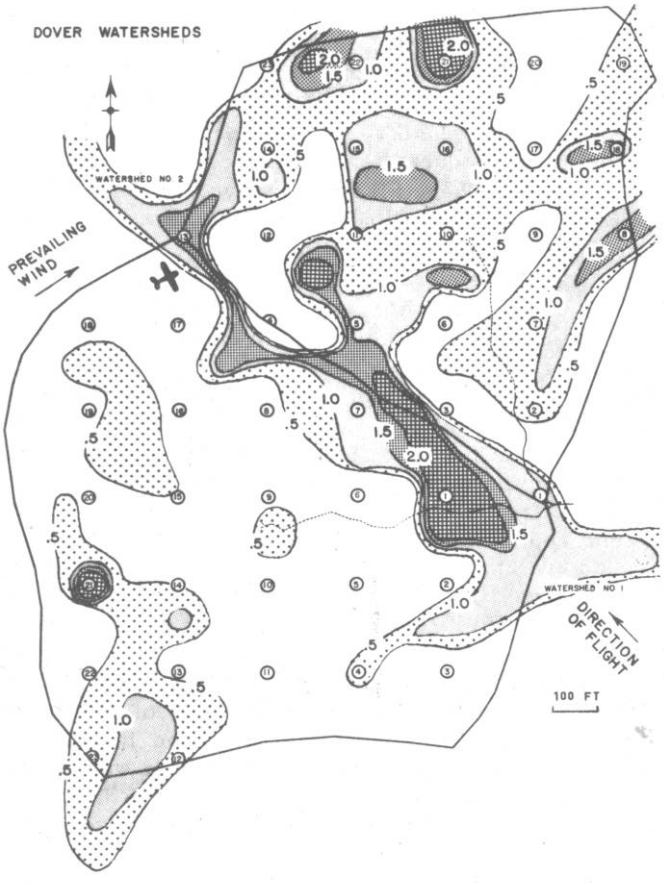

Fig. 3

Distribution of insecticide from one spray swath as measured in mean counts/min from 2 frosted glass discs at 100 ft intervals along the permanent 200 ft grid lines (Dover, Ohio)
The plane crashed where shown near the ridge top on 15 May 1962.

SPRAY ATOMIZATION AND DISPERSAL STUDIES

CARLETON et al. [18] state that: "The problem of measuring chemical distribution is one of our most critical because all other research hinges upon it. This will include highspeed measurement of particle size distribution, quantitative measurement of chemical distribution on the soil, and quantitative mass distribution of chemicals on plant surfaces. Until we have these methods available, all other research on pesticide application equipment will have a corresponding lack of precision."

HICKEY [19] contends that the ecological hazards of pesticides seem more closely related to the manner of use than to the volume used. Certainly the two are related, for, as CARLETON et al. [18] point out, since methods of application are relatively crude, control operators must use the simple expedient of applying more than minimum amounts of pesticides needed to control pests. These application excesses run up costs and residue hazards. For a single method of application (such as aerial, ground mist spray, etc.) it seems that hazard is related to volume used. However, total ecological hazards are related to volume and the number of habitat strata affected by the spray. Aerial application affects all strata and so presents the most complex ecological problems.

In forest aerial spraying, control effectiveness depends largely on two factors, timing and atomization (CARLETON et al. [18] and DAVIS et al. [16]. Forces acting on particles are conditioned by weather and time of day. Pest control generally is inversely related to particle size, but fine particles are difficult to place. An uneven, wasteful distribution is more common with the finer sprays. The balance of the factors of low cost, low gallonage, small particle size, increased effectiveness and total coverage is difficult to obtain. Failure to obtain such a balance has one alternative – ineffective control. Residue longevity can compensate for some gaps in control of motile pests, but insecticides such as malathion with short residual action and potentially small ecological hazards do not provide safeguards against non-treated gaps in area sprays.

In forest operations where large gallonage is required, fine droplet spraying is desired to improve the coverage and materially reduce the load required. Consequently the costs and the effects of weather and other factors on insecticide loss are reduced. Coarse atomization results in less vaporization and loss of spray, but finer atomization allows wider spray swaths and more uniform deposits.

Because the glass-disc method of determining spray distribution was considered highly reliable, attempts were made to correlate disc readings with more standard aerial spray assessment techniques. DAVIS and ELLIOTT [13] have summarized the methods normally used and their disadvantages. The assessment of water-emulsion sprays is more difficult than that of oil sprays, especially when droplet size measurement is desired. Following a suggestion from Dr. J. S. Yuill, United States Forest Service [25], assessment cards of woodbine enamel paper were tested. The Appleton Coated Paper Co., Appleton, Wisconsin, donated thirteen 25 × 30-in (63.5 × 76.2 cm) sheets of different colour enamel for testing. Water droplets from a hand atomizer were sprayed on test cards. There appeared to be no difference in spotting

by water and spray formulation. Emerald colour, 25×38–160 M, BS80 # bases, was judged best by a panel of three people to provide the most visible and distinct spots. Orange colour, 25×38–160 M, BS80 #, was next best. These two were far more suitable than any of the other 11 samples tested.

For the 15 May flight, two emerald cards, each 4×4 in (10.2×10.2 cm) were placed in previously installed wire holders which were set up on opposite sides of and 1 ft (30 cm) from each grid stake. The wire holders were those described by MAKSYMIUK [20] and were of 16-gauge wire which, though useable, was too light. The cards were put out less than one hour before the flight and removed 6 h later. Orange cards were used for the second flight. A light rain (unrecorded on the gauge) ruined 40% of the cards, most of them on area 2 following the 25 May 1962 application.

The cards at each location were picked up, numbered and placed in a plastic bag. The radioactivity of the cards was determined in a way perhaps more expedient than statistically sound. In the laboratory a circular disc of 1.25-in (2.98-cm) diam. and an area of 1.23 in^2 (7.93 cm^2) was cut at random from one quarter of each card, placed in a planchet and counted for radioactivity. This method seemed to meet the secondary interests of the study in comparing visual spray-deposit assessment methods with "known" radioactivity counts from the same cards or from other sampling devices.

Pirie's reagent (JEFFAY et al. [7] did not satisfactorily digest the paper as it did for preparing "biological samples." The planchet-size paper disc was considered an infinitely thick sample and was counted without further treatment. There are unknown absorption properties of the paper for the spray and the self-absorption of beta activity which influenced the readings.

Counting numbers of droplets and measuring droplet sizes on assessment cards is a tedious, difficult task with much possibility of error. While the comparison of cards with previously prepared "standards" is a desirable method (DAVIS and ELLIOTT [13]), no such standards were available for water emulsion or woodbine enamel paper.

THORNTON and DAVIS [21] recognized the difficulty of direct counts for measuring mass median diameter (MMD) and determined a sampling technique for counting card spots. We used a constant-area sampling intensity (1.23 in^2) which was well over that specified as desirable (0.5 in^2) when droplet sizes exceed 312 μm.

Cards were selected from both flights that had obvious dense spotting from the spray. Five were selected from the green cards of the one-swath flight; seven were selected from the orange cards. A circle of the same diameter (1.25 in) as that removed from each card was drawn with a compass slightly off-centre of the remaining card. The spots within the circle (7.7% of the total area of the card) were measured and tabulated.

Using a microscope with reticle we measured 20 droplets of malathion formulation suspended on the tip of a finely-drawn glass thread. Half of the droplets were placed on each of the two coloured assessment cards and allowed to dry. The spots were then measured with the same microscope scale and the measurements converted to microns. A spread factor or ratio of the mean spot size to the mean droplet size was obtained: (see Table III and MAKSYMIUK [20]):

Spread factor = mean spot diam. on card/mean spherical droplet diam.

TABLE III

COMPARISON OF METHODS OF ASSESSING SPRAY DROPLET SIZES AND ACTUAL RATES OF APPLICATION ON ENAMEL-COATED PAPER CARDS
Dover, Ohio 1962

Flight description card type and location	Median spot diameter B	Weighted mean spot diameter C	D_{max} A	Activity of card (counts/min)	Maximum activity of glass discs (counts/min)	Spread factor
Emerald: 15 May						
1-1	83	134	1300	14.0	8.0	
2-4	825	853	1300	0.	-	
2-16	495	539	2000	0.8	2.6	
2-17	825	701	1000	0	0.3	
2-23	110	124	600	0	0	
$\bar{x}$	468	467	1240	2.96	2.73	2.91
Orange: 25 May						
1-3	770	798	1700	95.4	129.9	
1-11a	1100	1062	3300	83.4	197.3	
1-11b	1100	1093	1800	110.7	197.3	

TABLE III (cont.)

Flight description card type and location	Median spot diameter B	Weighted mean spot diameter C	D_{max} A	Activity of card (counts/min)	Maximum activity of glass discs (counts/min)	Spread factor
1-14	495	619	1700	18.2	6.8	
1-16	1430	1368	2000	60.5	23.4	
1-22a	743	847	2000	235.6	381.8	
1-22b	963	914	2900	359.0	281.8	
$\bar{x}$	943	957	2200	137.5	188.3	2.66

The D_{max} reading was obtained from the entire card as described by MAKSYMIUK [22]. The five largest droplet spots were picked by eye, circled with a pencil, measured to the nearest 100 μm, and tabulated in descending order of magnitude. One spot, termed D_{max}, was the largest spot diameter with not more than 200 μm difference between it and the next smaller spot.

The mean spot size was determined by dividing the sum of the spot diameters by the number of spots. The median spot size is that one above or below which one-half the remaining observations fall. In the event of an even numbers of spots, the larger of the two median spots was selected as the median.

The conversion factors (D_{max} divided by the mean of the median spot diameters) of 2.6 for the emerald paper and 2.3 for orange paper are very close to 2.2 ± 0.08 reported by MAKSYMIUK [22]. A similar manipulation of figures provides almost identical conversion factors of 2.7 and 2.3 when the mean D_{max} is divided by the mean of the weighted mean spot diameters. The conversion factor makes use of the measured relationship between diameter and D_{max} to convert subsequent D_{max} observations to the more meaningful MMD:

$$\text{Estimated MMD} = \text{spot } D_{max} / \text{spread factor} \times \text{conversion factor}.$$

The estimated MMD for the 15 May 1962 flight was 177; for the 25 May flight, 360. In comparison the weighted mean diameter (WMD) was 161 and 360 for the two flights. This observation substantiates the use of D_{max} in estimating the MMD.

A comparison of the radioactivity of the cards and the maximum glass disc reading in counts/min yielded a significant correlation coefficient, r, of 0.97 for both spray flights. Either cards or glass discs therefore seem equally suitable for measuring isotope-labelled aerial spray.

The activity of the discs was 1.37 times that of the cards. Though not significant, the correlation coefficient of the D_{max} and card activity of the 25 May flight was 0.41. This seems to indicate that the system of D_{max}, though mechanically accurate, does not accurately estimate the actual amount of deposition. This observation can only be interpreted in the light of the relationship of desired droplet size for a particular spray application. The ratio of D_{max} to counts/min is 1:16. Our data indicate that D_{max} overestimates the actual quantity of deposition more frequently than it underestimates it.

ISLER and MAKSYMIUK [23] found that test helicopter flights provided an estimated 144 μm MMD compared to a measured MMD of 137. They classified this droplet size as within the "medium" atomization range of fixed-wing spray-planes used on forest insect-control jobs. DAVIS et al. [16] set the bounds of coarse, medium and fine atomization as 452, 291 and 151 μm, respectively. They found that a medium atomization and 150 MMD proved more consistently effective than coarser or finer atomization of DDT sprays for spruce budworm control. ISLER and THORNTON [24] found that a MMD of 150 μm provided the most efficient swath pattern for forest spraying. Finer sprays gave a wider, more uniform swath than medium sprays, but more spray was "lost." Coarse sprays resulted in narrow

swaths, reduced uniformity of distribution across the swath and high deposit peaks.

Figure 4 shows the variable pattern of spray distribution on the treated watershed and drift off the area as measured from spray-assessment cards.

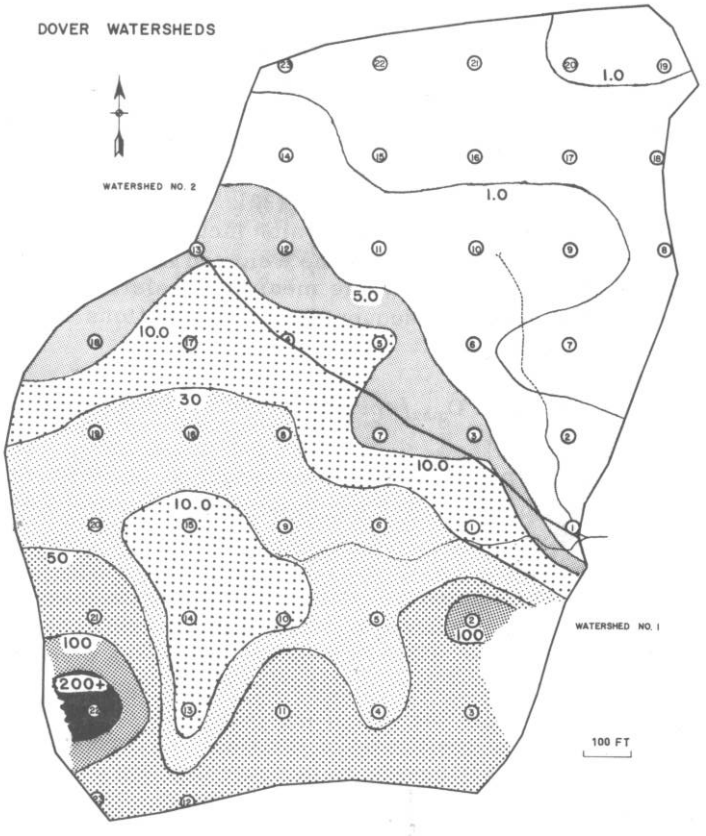

Fig. 4

Distribution of insecticide as measured in mean counts/min from enamel-coated spray assessment cards (25 May 1961 flight) (Dover, Ohio)

Figure 5 shows the amount of insecticide reaching the ground within two days after the application. Glass discs picked up on the date of spray were replaced and picked up two days later. This secondary "application" is of interest in co-ordinating aerial applications with foliation, with insecticide half-life and life histories of insects whose control is sought.

DISPERSAL

It is well-known that amounts of spray applied and deposited within an area vary widely. Ground assessment cards or discs present a picture

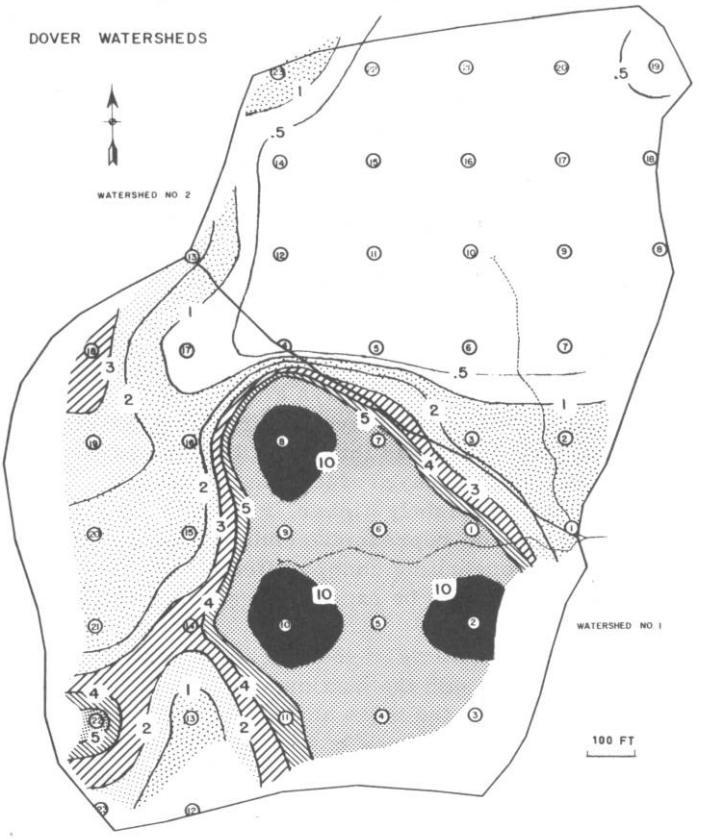

Fig. 5

Mean secondary dispersal of insecticide from rain-out 2 d after the 25 May application (Dover, Ohio) Counts/min from glass discs.

of the amount applied minus all the effects of the plane-to-card environment. The forest is the most complex of such environments and its effects, varying from direct interception to micro-turbulence, greatly influence the measured ground deposition.

An attempt was made to reduce numerically the effects of the forest on the glass-disc deposition readings. Over each of 43 sample points within the watershed, the vegetative density was estimated by two workers and a density rating given to each of the three major strata; tree, shrub, and herbaceous. A rating of 1 was equivalent to "heavy" or 70-100% canopy closure; 2, moderate, 30-70%; 3, light, 1-30%; and 4, canopy absent.

Within the forest, each layer is not believed to have equivalent effect on spray interception and re-distribution. The open area between strata canopies is variable: tree to shrub, approximately 40 ft; shrub to herbaceous, approximately 4 ft; herbaceous to ground, approximately 1 ft.

We believe that the greater the distance between strata canopies, the move homogeneous will be the unintercepted spray. On the basis of this as-

sumption, each vegetative strata density was multiplied by the following factors: tree, 0.18, shrub, 0.22, and herbaceous, 0.60, to give a truer picture of the influence of the density of each strata on the insecticide finally deposited. Vegetative density observations can range from 1, 1, 1 (all layers heavy) to 4, 4, 4 (all layers absent). The density influence rating (DIR) therefore ranges from 0.33 to 1.33. It is expected that the higher ground disc readings will positively correlate with higher DIR. Lack of correlation is the influence of variable amounts applied above the canopy. When DIR was compared with the maximum disc reading, a significant correlation coefficient of 0.32 was obtained; with mean disc readings, r equalled 0.28, but was not significant. A slight negative correlation $r = -0.051$ was obtained between DIR and maximum disc readings on the neighbouring control area where drift occurred.

From glass discs left out two days after spraying, a negative correlation would be expected, since leaves intercepting the higher quantities would "apply" the spray. Such was the case. DIR compared with maximum glass-disc readings 2 d after spray produced an r of −0.015. The correlation of DIR with the mean was slightly higher, $r = -0.07$. Neither correlation was significant, but they do represent trends. This tends to support the previous assumptions concerning canopy effects on atomization, since the post-spray "rain-out" would not be expected to approximate the same pattern as the aerial application.

The total ecological effects of insecticide deposition are believed to be more closely correlated with the maximum reading on the sampling units (card or glass disc) than with the mean of the readings at any one point. The application within a 1-ft-square area is assumed to be uniform. Spray assessment units on the ground would be expected to receive nearly identical deposits. Such is not the case. At one sampling point in watershed 1, three glass discs within a 1-ft-square area received 217.4, 42.7, and 16.3 counts/min of insecticide from the 25 May flight. If A is the amount applied to the top of the tree canopy, D the amount deposited in a 1-ft-square area, and I the amount of spray intercepted by trees, evaporated, etc., then $D = A - I$. If A is assumed constant for a given area, then D varies with I, not A, and therefore the best estimate of the A is the maximum D reading or the D with the minimum I influence. The best estimate of the actual amount of spray applied to a given area is obtained by:

$$\frac{\text{Maximum sample unit reading per sampling station}}{\text{DIR}} \times \frac{\Sigma\ \text{DIR}}{\text{Number of stations}}$$

PATTERNS OF DISTRIBUTION

1. Above the forest

Helium-filled balloons, on which were glued glass discs, were tethered above the centre of the treated area. Counts recorded were 277.0, 217.6, and 206.1 counts/min. This represents the approximate rate of deposition of 2 lb/acre of malathion on top of the forest canopy. Data from air-sampling devices near the study area have not yet been analysed.

2. Within the tree layer

Glass discs were suspended in pulley-attached ropes within six trees in a transect through the forest. Discs were at intervals of 5 ft. The uppermost 10-15 ft of the trees were not sampled. At no position within the tree canopies were readings as high as those recorded on the balloons over the forest. The upper 10-20 ft of canopy apparently intercepts over 50% of the spray. The remaining spray is intercepted irregularly throughout the tree layer (see Fig. 6). There is no apparent correlation between height above the ground and amount of spray (counts/min) delivered.

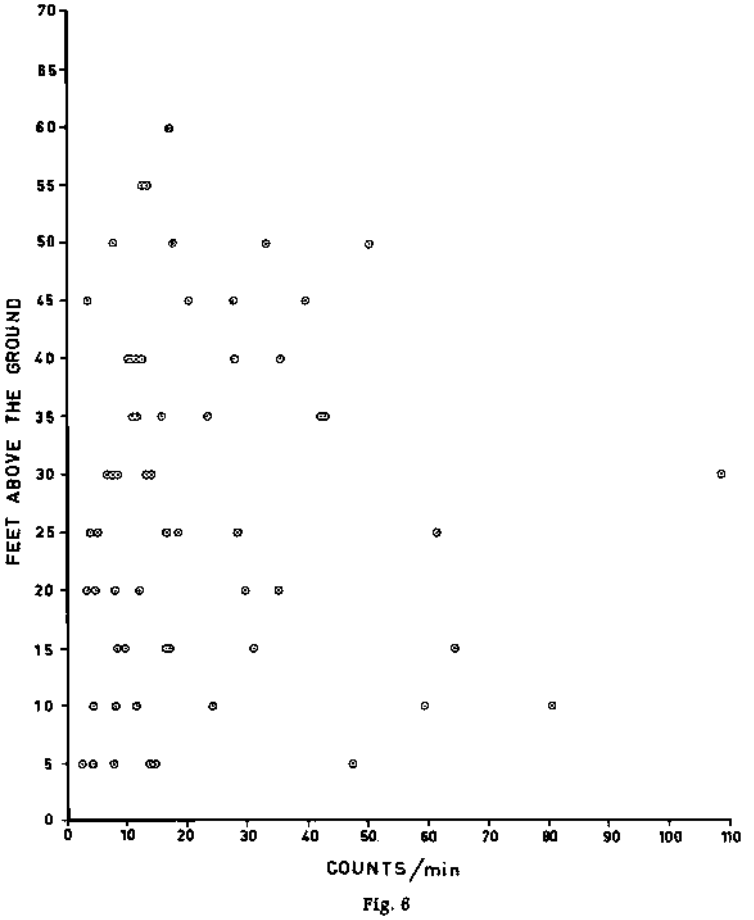

Fig. 6

Scatter diagram of deposition of insecticide (counts/min) on glass discs suspended at 5-ft intervals within 6 tree canopies within the forest (Dover, Ohio 1962)

3. Within the shrub layer

In an effort to discover the distributional pattern of insecticide within and under the shrub layer, glass discs were again utilized. Frosted glass

TABLE IV

DISTRIBUTION OF MALATHION IN COUNTS/MIN UNDER SMALL SHRUB OR SAMPLING CANOPIES AS MEASURED FROM GLASS DISCS
Dover, Ohio, 1962

Shrub or sampling species	Distance from ground (in)									
	6	12	18	24	30	36	42	48	54	60
Spicebush *Lindera benzoin*	13.7	7.8	5.7	10.4	13.8	31.1	9.4	17.9	40.0	28.9
Tulip poplar *Liriodendron tulipifera*	0.0	19.8	6.1	14.3	6.2	6.7	9.4	14.5	35.4	12.7
Red maple *Acer rubrum*	9.9	13.3	3.9	5.2	24.6	7.3	38.7	6.0	5.7	14.6

discs were affixed with rubber cement to blocks spaced 6 in (15.24 cm) apart on a 1 in × 3 in × 8 ft pine board set at a 45° angle to the ground. Three of these devices were placed in the centre of watershed No. 1 on 23 May. Each was set up as a tripod under (1) an 8-ft maple coppice, (2) a 6-ft spicebush and (3) a 10-ft tulip poplar sapling. The base of the pine board was placed on the ground at the base of the shrub. The technique was designed to sample the insecticide passing through the segments of the shrub crown radius before the droplets reached the forest floor, where they might be affected by ground winds. In Table IV it can be seen that the irregular pattern of spray dispersal noted within the tree layer prevails. There is a slight tendency for larger amounts to be deposited above 1 m.

4. Within the litter

Field G-M counter readings presented in Table V indicate that radioactivity and thus malathion at 2 lb/acre does not penetrate more than four layers of dead A_1 horizon leaves. The background was about 0.04 mr/h.

TABLE V

PORTABLE FIELD COUNTER READINGS OF RADIOACTIVITY OF LEAF AND SOIL LAYERS Dover, Ohio 1962*

Description	Plot 2 (mr/h)
First layer	To 0.2
2nd layer	To 0.17
3rd layer	0.09
4th layer	0.05
5th layer	0.04
6th layer	0.03
Layers indistinct ¼" below leaf layers	0.01
Soil - 1 in	0.04 - 0.05
Soil - 3 in	0.03 - 0.04
Soil - 4 in	0.01 - 0.02

* Readings taken with a Model P-2612 N.C. thin-window G-M tube, 1.4 mg/cm², under a point near stake 20, plot 2, from 10.0-12.0 a.m. 15/9/1961.

In another experiment glass discs were placed under varying numbers of leaves held in place with a wire pin. The discs were removed periodically and counted. The results, much more sensitive than the G-M readings, are shown in Table VI. The results are variable because of wind blowing the leaves, even though fastened, molestation by animals and continued "rain-out" of insecticide from the overhead tree canopy. These data seem to indicate that higher disc contamination occurs under fewer leaves. The data are not reliable enough to differentiate absorptive properties of leaves for the insecticide.

TABLE VI

DISTRIBUTION OF MALATHION OR ITS METABOLITES THROUGH VARYING NUMBERS OF LEAVES ON THE FOREST FLOOR
Dover, Ohio, 1962

		27-5-62	6-6-62	15-6-62	13-7-62	7-8-62
Plot 1(1-21) Number of hickory leaflets	0	-	3.2	4.4	6.6	-
	1	-	5.5(2.3)	3.5	6.1	5.8
	2	-	2.6(7.3)	4.2	3.2	4.0
	3	-	3.1(2.8)	7.2	3.6	3.8
	4	-	6.2(2.6)	-	-	4.4
	5	-	1.8	3.3	-	3.4
Plot 2 near (1-14) Number of beech leaves	0	7.2	3.8	10.0	8.4	-
	1	-	9.6	4.7	4.6	-
	2	1.5	3.9	-	2.1	-
	3	3.0	3.7	3.1	3.1	-
	4	-	2.6	3.6	2.0	-
	5	-	4.4	-	3.4	-
Amount of rainfall since spray		0.09	2.5	3.25	4.7+	4.7+

5. Within the soil

The previous data show that the absorptive properties of the forest litter prevent malathion from entering the soil. This was also borne out by contamination readings on marked stakes driven into the soil for this purpose and glass discs placed in a pit face.

An airplane crash on 15 May provided an unusual opportunity to study the movement of a large (30-40 gal) quantity of radioactive insecticide in a Welston silt loam soil. Figures 7 and 8 show above- and below-ground deposition and movement of the insecticide. Soil core samples were taken with a 1-in diam. King tube in a gridded plot around the crash site. The activity of each one-foot core sample was determined, using a modification of the previously described method. Cross-sectional distribution data are now being studied and will be presented in a later paper. It appears that, even in large quantities, the formulation of malathion moves relatively slowly. The majority of the solution activity remained at the site of deposition as evidenced by low counts delineating the movement boundaries.

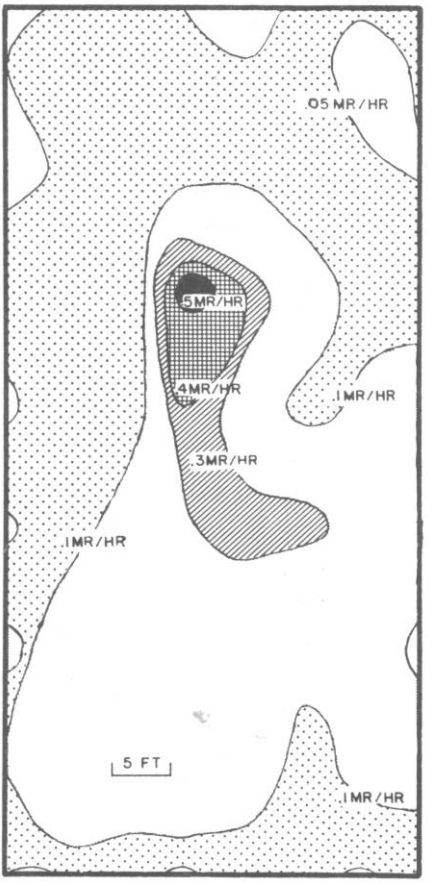

Fig. 7

Surface ground contamination in mr/h in a plot around the crashed Piper J-3 spray plane 10 d post spray. (Dover, Ohio 1962)

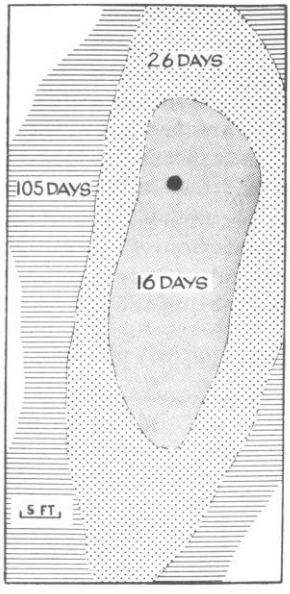

Fig. 8

Internal movement (3-7 ft) of 30-40 gal of radioactive malathion formulation in Welston silt loam soil following a spray plane crash (Dover, Ohio 1962)

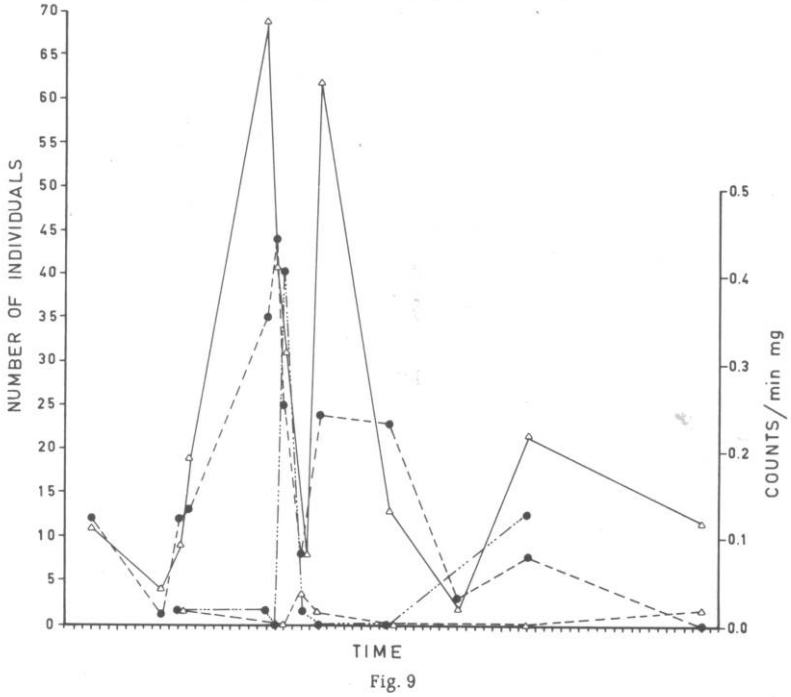

Fig. 9

A comparison of light-trap samples of the adult Geometridae populations with radioactivity of the sample in counts/min mg by days (Dover, Ohio 1962)

FAUNAL EFFECTS

Mammal, reptile and amphibian populations, as suggested by our census indices, were unchanged by the application. One aspect of bird behaviour was altered for three days: they stopped singing in the treated area. Flycatchers, wood thrushes and warblers continued nesting. Young in nests under observation on the treated area were reared successfully.

Insect population losses were great. Analyses of these data are not complete. Examples of the type of data inspection being made are Fig. 9 of the Geometridae and Fig. 10, the Nitidulidae.

The relationship of the population size to radioactivity in the Nitidulidae sampled with molasses traps is only correlated for about six days, after which there is a decline in activity regardless of population fluctuations. Further studies on life history and feeding habits will be made to attempt to explain this observation.

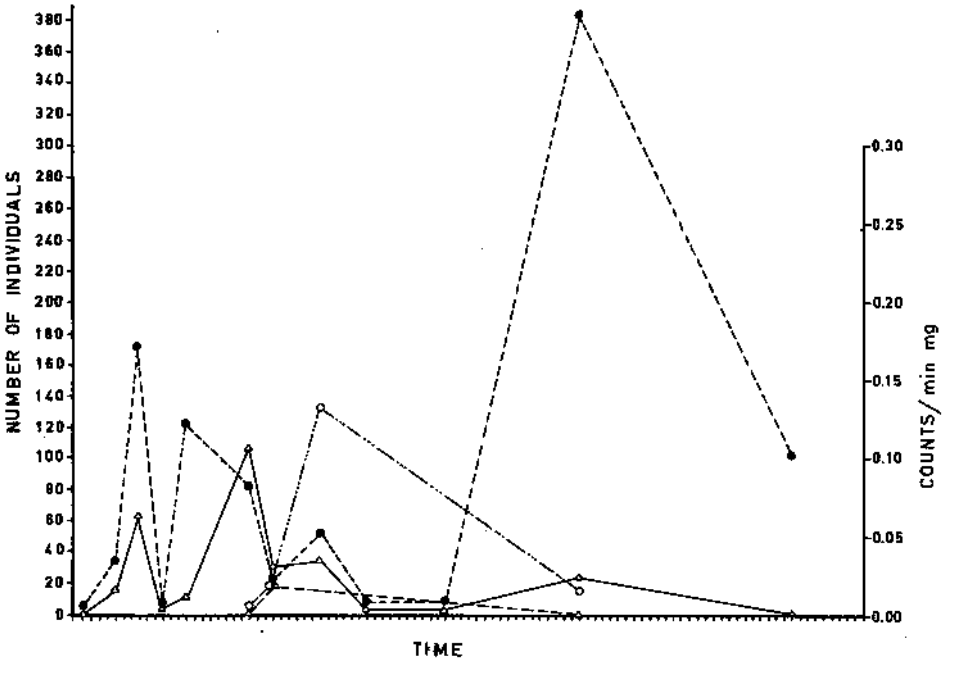

Fig. 10

A comparison of molasses-trapped adult Nitidulidae with radioactivity of the sample in counts/min mg by days (Dover, Ohio 1962)

	Watershed 1	Watershed 2
Number of individuals	-●--●	-Δ——Δ-
Counts/min mg	-O˙˙O-	-Δ·····Δ-

In the case of adult Geometridae sampled by light traps, two peaks are evident in the radioactivity measurements. These occur immediately after the application and again following an apparent population decline. Again, analyses of these findings are incomplete.

No effects were observed on soil micro-fauna, cryptozoa or earthworms.

SUMMARY

Malathion-S^{35} was applied to a 23.4-acre Ohio hardwood forest in May 1962. Faunal population changes from previous-year norms were studied, as were the movements of the insecticide within the ecosystem.

Studies of spray distribution are reviewed. Wide variance in lateral, horizontal and vertical distribution of the insecticide formulation in the forest are described and illustrated.

Water emulsion assessment cards were developed and calibrated. The accuracy of the D_{max} system of estimating MMD of atomization was substantiated. The D_{max} system, though mechanically accurate, most often overestimates the actual amount of deposition. A method is presented for computing the best estimate of the actual amount of spray applied to a given area.

Preliminary faunal data inspection indicates no adverse effects of the application except to insects.

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DISCUSSION

W. KLOFT: I think some of the distribution of your labelled insecticide may be due also to secondary sublimation of the insecticide into the air, and to further distribution by air flows. We have done similar studies, on a much smaller scale, with radioactive-labelled organic insecticides such as, for example, Thiodan. This has very high vapour pressure and can be sublimated from the insect, after a rise in body temperature following intoxication, into the air, and also spread by the air.

The insecticide might be secondarily distributed in this way in your case too. The dark surfaces of plants, bark for instance, could be heated by the sun during the day so as to show a secondary sublimation.

T. J. PETERLE: That is possible. The glass discs which we had distributed vertically in the canopy and horizontally in the forested area were picked up approximately seven hours after the aerial application, so some sublimation may have occurred in the seven hours from early morning until we allowed the insecticide to settle out fully and we felt free to go into the area.

J. E. CASIDA: Did you take any precautions to determine whether your S^{35} was as malathion or as degradation products of malathion?

T. J. PETERLE: We did check some of the metabolites of malathion later with the labelled material and found that the sulphur seemed to be distributed very well in the degradation products. Exactly which those are or how many contain actual sulphur I am uncertain, but in doing paper chromatography studies we found the sulphur was fairly well distributed.

J. E. CASIDA: Do you think that the insects which you show in your graphs were picking up malathion?

T. J. PETERLE: Initially yes, because malathion of course has a residual action of about ten days. After that it could have been the metabolites of just S^{35}.

J. E. CASIDA: You indicated that you were going to continue to sample this year? Would you be studying malathion?

T. J. PETERLE: No, I would not expect to find malathion this year. We shall be checking for S^{35} residues.

J. E. CASIDA: Malathion hydrolyses rather quickly at the carboxyl-site when exposed on the surface of plants, and it might be difficult therefore to interpret some of your results.

M. AROYO VARELA: Did you take advantage of the opportunity to study the influence of the insecticides on the insect parasite fauna? I would be interested to know if you did, and if so, what were your conclusions.

T. J. PETERLE: Yes, we used many sampling techniques, and we can sort out the predaceous insects in our data and examine them as the residual quantity builds up for a period of days. The data from specific insect families is available but it has not been analysed from the standpoint of prey-predator relationships. This will be included in the final publication

E. E. TURTLE: Are any of the birds or mammals collected in and around the sprayed site being analysed for S^{35}?

T. J. PETERLE: Yes. We have data on a wide spectrum of the vertebrate fauna. Since this Symposium was entomologically oriented, we have excluded those data from the paper. The vertebrate residue data will be published in the final report.

E. E. TURTLE: These data would be very valuable for assessing the effects of insecticides less readily metabolized than malathion.

NOTE PRÉLIMINAIRE SUR L'UTILISATION DES RADIOISOTOPES DANS L'ÉTUDE DES PARASITES DU COTONNIER EN AFRIQUE

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Abstract — Résumé — Аннотация — Resumen

PRELIMINARY NOTE ON THE USE OF RADIOISOTOPES TO STUDY SOME COTTON-PLANT PESTS IN AFRICA. The larva of Diparopsis watersi Roth is harmful to the cotton-plant, destroying the flower and the boll. This noctuid, which is practically monophagous, passes the quiescent season either in the ground in the chrysalid-at-diapause form (from 10 November onwards) or in continuing generation (polyvoltine) on plants left standing. Laboratory studies have served to elucidate the main mechanisms initiating and terminating diapause.

In the field, the rapid multiplication of the pest during the crop period is due both to univoltine individuals hatching out towards April-May and to non-diapause generations. The respective roles played by these two different sources need to be determined fairly accurately in order to decide which methods of control are appropriate: uprooting of the cotton-plant, destruction of the pupae at diapause in the ground, bringing the crop forward, etc. Radioisotope labelling of larvae coming to maturity at critical periods should make it possible to tackle this problem at the practical level.

Preliminary experiments for determining simple labelling techniques have been carried out at Tikem (Republic of Chad). In a first trial, P^{32} in atomized solution was applied direct to the foliage of young cotton-plants. Despite the effects of rain, absorption by the plant reached about 10% in a few hours. Radioactivity is not retained by various phyllophagous larvae (Sylepta derogata, Prodenia litura), but those feeding on the fruit-bearing parts of the plant (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) are easily detectable three months after the application.

In a second trial, P^{32} and S^{35} were applied to old cotton-plants immediately before the natural time for Diparopsis to enter diapause. The overall findings give hope of being able to distinguish without too much difficulty, among chrysalid populations taken from the ground, those which fed on the cotton-plant before labelling, and hence which underwent a diapause.

A control experiment will now be required, consisting of labelling, during the season, cotton-plants in a field where cotton was also grown the previous year; this time non-diapause chrysalids will be labelled. Similar observations are also being performed on a number of other cotton-plant pests.

NOTE PRÉLIMINAIRE SUR L'UTILISATION DES RADIOISOTOPES DANS L'ÉTUDE DES PARASITES DU COTONNIER EN AFRIQUE Le chenille de Diparopsis watersi Roth est nuisible au cotonnier en détruisant fleurs et capsules. Cette noctuelle, pratiquement monophage, passe l'inter-saison, soit dans le sol sous forme de chrysalides en diapause à partir du 10 novembre, soit par des générations continues (polyvoltines) sur les plants non arrachés. Des études de laboratoire ont permis d'élucider les principaux mécanismes déclenchant la diapause et y mettant fin.

Dans la nature, les pullulations en cours de culture s'établissent à partir d'individus univoltins venant à éclosion vers avril-mai aussi bien que des générations sans diapause; la participation respective de ces deux souches différentes devrait être déterminée assez exactement pour connaître les moyens de lutte appropriés: arrachage du cotonnier, destruction des pupes en diapause dans le sol, avancement de la campagne, etc. Le marquage par des radioisotopes des chenilles venant à maturité aux époques critiques devrait permettre de serrer ce problème sur le plan pratique.

Des expériences préliminaires ont eu lieu à Tikem (République de Tchad) en vue de déterminer des techniques de marquage simples. Dans un premier essai, ^{32}P a été appliqué en pulvérisation aqueuse directe sur le feuillage de cotonniers jeunes. Malgré la pluie, l'absorption fut de 10% environ par la plante en quelques heures. Diverses chenilles phyllophages (Sylepta derogata, Prodenia litura) ne retiennent pas de radioactivité, mais les chenilles qui se nourrissent d'organes fructifères (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) sont facilement détectable trois mois après l'application.

Dans un deuxième essai, ^{32}P et ^{35}S ont été appliqués sur des cotonniers âgés, juste avant la période de mise en diapause naturelle de Diparopsis. Les résultats d'ensemble laissent espérer que l'on pourra sans trop de difficultés distinguer, parmi les populations de chrysalides prélevées dans le sol, celles nourries sur le cotonnier avant qu'il ne soit marqué, donc qui auront subi une diapause.

Une contre-expérience sera ensuite nécessaire; elle consistera à marquer, en cours de saison, les cotonniers d'un champ ayant déjà porté la même culture l'année précédente, les chrysalides sans diapause étant cette fois marquées. Des observations annexes sont également en cours sur divers autres parasites du cotonnier.

ПРЕДВАРИТЕЛЬНЫЕ ЗАМЕЧАНИЯ О ПРИМЕНЕНИИ РАДИОИЗОТОПОВ ДЛЯ ИЗУЧЕНИЯ НЕКОТОРЫХ ВРЕДИТЕЛЕЙ ХЛОПЧАТНИКА В АФРИКЕ. Гусеница Diparopsis watersi (Roth) наносит вред хлопчатнику, уничтожая цвет и коробочку. Эта, практически, монофаговая совка проводит межсезонный период либо в земле в виде куколки в состоянии диапаузы, начиная с 10 ноября, либо в виде новых (поливольтинных) поколений на не выкопанных из земли растениях. Лабораторные исследования позволили выяснить основные механизмы возникновения и прекращения диапаузы.

В природе интенсивное размножение при разведении культур происходит у одних особей с одним поколением в год, выплансивающихся к апрелю-маю, у вторых - появление поколений без диапаузы; относительную роль этих двух различных ветвей особей следовало бы точно определить для выбора метода борьбы: выкорчевка хлопчатника, уничтожение куколок в состоянии диапаузы в земле, сокращение сроков сельскохозяйственной кампании и т.д. Мечение радиоизотопами гусениц, которые в критический период достигают зрелости, должно сузить эту проблему в практическом плане.

В Тикеме (Республика Чад) были проведены предварительные опыты для определения простых методов мечения. В первом опыте был использован P^{32} при прямом водном обрызгивании листьев молодого хлопчатника. Несмотря на дождь, поглощение растением в течение нескольких часов составляло около 10%. Различные филлофаговые гусеницы (Sylepta derogata, Prodenia litura) не накапливают радиоактивности, однако питающиеся плодоносными органами гусеницы (Heliothis armigera, Earias insulana, Diparopsis watersi, и т.д.) легко обнаруживаются спустя три месяца после применения этого метода.

Во втором опыте P^{32} и S^{35} были использованы для взрослых кустов хлопчатника как раз накануне периода естественного перехода Diparopsis в диапаузу. Результаты позволяют надеяться на то, что удастся без излишних трудностей выявить среди изъятых из земли куколок тех, которые питались хлопчатником до его мечения и которые поэтому пройдут диапаузу.

Необходимо проведение обратной проверки, которая состоит в том, чтобы в течение сезона пометить хлопчатник на поле, уже находившемся под этой культурой в прошлом году. В данном случае куколка без диапаузы оказывается меченой. В настоящее время проводятся также дополнительные наблюдения за другими вредителями хлопчатника.

NOTA PRELIMINAR SOBRE EL EMPLEO DE RADIOISÓTOPOS EN EL ESTUDIO DE PARÁSITOS DEL ALGODONERO EN ÁFRICA. La oruga de Diparopsis watersi Roth es perjudicial para el algodonero, pues destruye sus flores y cápsulas. Este noctúido, prácticamente monófago, pasa el período interestacional bien en el suelo en forma de crisálidas en diapausa a partir del 10 de noviembre, bien por generaciones continuas (especies que se reproducen varias veces al año) en las plantas sin arrancar. Los estudios de laboratorio han permitido dilucidar los principales mecanismos que determinan el comienzo y el fin de la diapausa.

En la naturaleza, las pululaciones durante el cultivo provienen de individuos de una sola generación al año, que alcanzan la eclosión hacia abril o mayo, así como de generaciones sin diapausa. Debería averiguarse con suficiente exactitud el papel que desempeñan estas dos cepas diferentes, para determinar los medios de lucha más apropiados: arranque del algodonero, destrucción de las ninfas durante la diapausa en el suelo, anticipación de la campaña, etc. La marcación radioisotópica de las orugas que alcanzan la madurez en las épocas críticas permitiría seguramente abordar este problema en el plano práctico.

En Tikem (República del Chad) se han realizado experimentos preliminares para preparar técnicas sencillas de marcación. En el primer ensayo se aplicó ^{32}P en pulverización acuosa directa sobre el follaje de algodoneros jóvenes. Hay orugas filófagas (Silepta derogata, Prodenia litura) que no retienen la radiactividad, pero las que se alimentan de órganos fructíferos (Heliothis armigera, Earias insulana, Diparopsis watersi, etc.) se pueden detectar fácilmente tres meses después de la pulverización.

En el segundo ensayo se aplicaron ^{32}P y ^{35}S a algodoneros viejos, inmediatamente antes de comenzar el período de diapausa natural de Diparopsis. Los resultados obtenidos hacen pensar que no será muy difícil distinguir, entre las poblaciones de crisálidas recogidas del suelo, las que se han nutrido en el algodonero antes de ser marcado, es decir, las que habrán sufrido una diapausa.

Seguidamente será preciso un experimento comprobatorio, consistente en marcar en plena temporada a los algodoneros de un campo que haya tenido el mismo cultivo que el año precedente, pero limitándose esta vez a las crisálidas sin diapausa. También se están haciendo estudios complementarios sobre otros parásitos del algodonero.

Parmi les nombreux parasites qui infligent chaque année des pertes considérables aux cultures cotonnières en Afrique, la chenille d'une noctuelle, Diparopsis watersi, est particulièrement néfaste dans les zones sahéliennes et subsahéliennes qui s'étendent depuis le Mali jusqu'au Tchad.

Cette chenille a fait l'objet de longues recherches de la part d'auteurs anglais en Nigeria et au Sudan, et la Station IRCT de Tikem, au Tchad, a fait porter son programme sur ce parasite. Les résultats obtenus par Galichet ont complété ou éclairci nombre de points sur la biologie de l'insecte et contribué à une meilleure connaissance des facteurs de la diapause.

En effet, Diparopsis offre deux particularités biologiques importantes. Tout d'abord, c'est un insecte à peu près strictement monophage, admettant les diverses espèces de cotonnier comme nourriture constante, et ne pouvant que très occasionnellement et très temporairement vivre aux dépens de quelques malvacées très proches. Dans la nature par conséquent, le maintien de Diparopsis dépend strictement de la présence d'une espèce ou d'une autre du genre Gossypium.

En second lieu, cette noctuelle possède, comme bien d'autres lépidoptères et bien d'autres arthropodes, un stade de repos plus ou moins prolongé, touchant le stade pupal (chrysalide), auquel le déterminisme précis donne un caractère de véritable diapause. Cette phase a lieu dans le sol, à quelques centimètres seulement de la surface. Toutefois, tous les individus ne sont pas obligatoirement touchés par cette diapause; au début de la saison, les conditions extérieures (température et aussi photopériodisme, hygrométrie, etc.) ne sont pas favorables à la mise en diapause, et seuls quelques individus la subissent. Au début du mois de novembre, la température nocturne s'abaisse en-dessous de 16° tandis que la température diurne reste encore élevée (supérieure à 32°) et en même temps la durée du jour diminue de façon assez rapide; à cette époque une forte majorité de chenilles entre en diapause, pas la totalité cependant.

Galichet a pu émettre l'hypothèse de l'existence de deux races de Diparopsis se trouvant en mélange dans toute la zone tchadienne, l'une insensible à la diapause et évoluant donc sans interruption d'une campagne à l'autre, l'autre pouvant se mettre en diapause à une époque déterminée et attendant jusqu'au printemps sous forme de chrysalide la culture suivante. Au laboratoire, des élevages de souches différentes auraient donné une première confirmation de cette hypothèse.

Mais en se plaçant sur le plan pratique, il est un problème que les études de laboratoire, en milieu artificiellement conditionné, ne sauraient résoudre : c'est le problème de la participation respective des générations venues de diapause et des générations qui au contraire se sont succédé sans interruption depuis la campagne précédente. En effet, la lutte doit s'orienter de manière à briser le cycle au moment le plus favorable; dans le premier cas, c'est la phase chrysalide en diapause dans le sol, et on procédera par des interventions agricoles (labour, scarifiage, etc.) sur le champ venant de porter du coton, ou encore par un assolement tel que les champs de

l'année soient très éloignés des emplacements ayant porté du coton l'année précédente; dans le second cas, ce sont les plants de cotonniers non arrachés en fin de campagne qui hébergent les chenilles pendant la morte saison théorique que l'on devra pourchasser complètement.

Il faudrait donc déterminer dans un champ cultivé en coton deux années de suite, quel est le pourcentage de chrysalides en diapause et celui de chrysalides sans diapause au début de la deuxième campagne. Comme il s'agit de races physiologiques ne pouvant se reconnaître par aucun détail anatomique externe ou interne, on se trouve face à une difficulté pratiquement insurmontable jusqu'à présent.

En faisant intervenir le marquage par les radioisotopes, on a pensé tenir un moyen théoriquement plus simple de résoudre cette question. En effet, si l'on peut marquer la plante pendant un temps donné, les chenilles ayant évolué sur cette plante seront elles aussi marquées, ainsi que les chrysalides qui en résulteront plus tard, tandis que les chenilles ayant donné des chrysalides avant que la plante ne soit marquée ne seront pas actives. On peut donc avoir un datage relatif de ces chenilles - et reconnaître celles appartenant aux générations sans diapause.

Ce datage peut être réalisé dans un autre sens si l'on peut trouver un isotope possédant une vie assez longue : on reconnaîtra alors les chrysalides qui ont vécu la campagne précédente sur des plants marqués.

C'est en vue de déterminer les conditions préliminaires d'une telle recherche que nous avons réalisé les premières expériences de marquage, à la Station de Tikem (Tchad), dans un programme de coopération avec la Section «Biologie» du CENS.

Les questions préalables à résoudre étaient celles-ci :

- Peut-on procéder par pulvérisation directe sur le feuillage afin de diminuer autant que possible la contamination du sol, qui estomperait en les allongeant les limites du marquage?
- Quelles substances employer qui soient absorbées par la plante et assimilées par l'insecte, sans présenter de danger trop grand à l'usage dans les conditions très rustiques où l'on se trouve?
- Quelles durées valables de marquage obtiendra-t-on, et comment le diagnostic sera-t-il obtenu avec le plus de précision?

PREMIER ESSAI PRÉLIMINAIRE

Dans une première expérience on a choisi le ^{32}P pour des raisons évidentes de prix de revient, de facilité d'obtention et de repérage de probabilité d'absorption aisée par le feuillage, etc. Les inconvénients auxquels on s'attendait a priori étaient : vie assez limitée, manipulation en plein air ayant un certain risque, etc.

L'application a été réalisée le 13 août dans les conditions suivantes :

Matériel

- Equipement de protection : scaphandre autonome avec masque respiratoire et appareil générateur d'oxygène «Feuzy», gants et surchausses de caoutchouc;
- Compteur portatif pour le contrôle et la décontamination;

- Réserve d'eau et seaux pour lavages;
- Pulvérisateur type Vermorel (fixé sur le ventre en raison de l'appareil respiratoire fixé sur le dos) et lance de pulvérisation;
- Seringue et récipients pour la manipulation du ^{32}P.

Conditions d'application

Attendre que la rosée ait presque complètement disparu, pour éviter le ruissellement, mais aussi opérer avant que le vent ne se lève, ce qui aurait présenté un accroissement de danger pour l'opérateur et diffusé la substance nors des limites tracées. Bien qu'on se trouva à cette époque en pleine saison des pluies, pluies après l'exécution, la chance a permis que l'application ne soit lessivée que trois jours après.

Commencés à 9 h 30, les préparatifs ultimes et le traitement étaient terminés à 9 h 50.

4 billons de 20 m avaient reçu 8 l de solution contenant environ 9 mc. (Le 7 août, jour du contrôle de l'activité au Laboratoire de Saclay, l'échantillon envoyé donnait 13 mc.)

Les obstacles rencontrés au cours de cette application furent :

- la gène apportée par les hauts billons dans la marche rendue déjà pénible par le scaphandre et l'appareil respiratoire;
- l'encombrement du pulvérisateur de grand volume porté en position très incommode sur le ventre;
- la température et surtout l'hygrométrie très élevées s'établissant rapidement à l'intérieur du scaphandre et rendant particulièrement pénible le travail dans un soleil déjà chaud.

Malgré celà, l'opération eut lieu jusqu'au bout sans incident. Une équipe dirigée par un européen procéda ensuite aux premières décontaminations sur place. La décontamination fut complétée ensuite à l'aide du compteur gammamètre; les instruments et accessoires ne pouvant être décontaminés furent immergés dans le vaste lac de Tikem ou jetés dans les galeries d'une termitière profonde.

On peut suggérer quelques améliorations de détail à ce modus operandi. En particulier nous pensons qu'un système de pulvérisation comportant une bouteille de Freon comme source de pression allégerait notablement le travail physique de l'opérateur.

Ramassages de chenilles

On « enrichissait » la parcelle en y apportant les chenilles de tous âges rencontrées au cours des autres recherches entomologiques faites par la Section. Puis on ramassait ces chenilles aux dates suivantes : 12 septembre, 24 septembre, 1er octobre et 8 octobre. Elles avaient donc passé, sur les plants traités, des temps variables, mais au moins égaux à 24 h.

Ces chenilles mises en alcool furent expédiées en quatre lots au Laboratoire d'applications agronomiques du CENS où, grâce à l'obligeance des collaborateurs, leur activité fut déterminée les 22-23-24 et 25 octobre. Pour cela le tégument est coupé longitudinalement et la chenille est ouverte, les viscères sont dilacérées avec des épingles, et le tout est passé à l'étuve

jusqu'à dessication. On a vérifié que l'alcool qui a servi au transport ne retenait aucune activité appréciable.

Les exsiccats individuels sont ensuite pesés, et le tableau des résultats bruts est ainsi obtenu.

Pour utiliser ces résultats, il convient d'abord d'établir des corrections puisque l'activité a décru notablement entre la date de récolte et la date du comptage.

On obtient la valeur de l'activité le jour de la récolte en multipliant les chiffres de comptage respectivement par 6,6 4 3,10 et 2.

Le tableau I donne des valeurs moyennes, établies par classes, de 30 en 30 mmg.

TABLEAU I

VALEURS MOYENNES DE L'ACTIVITÉ

	0 - 30	30 - 60	> 60
1er lot (12.9)			
Nombre chenilles	16	3	3
Poids moyen	12	42	72
Activité/larve	5	10,1	10,2
" corrigée	33	66,6	67,3
Activité/mmg	0,44	0,24	0,19
" corrigée	2,9	1,58	1,18
2e lot (24.9)			
Nombre chenilles	4	4	10
Poids moyen	5	50,1	83,2
Activité/larve	9,7	10,7	5,88
" corrigée	38,8	42,8	23,5
Activité/mmg	1,86	0,20	0,07
" corrigée	7,4	0,8	0,3
3e lot (1.10)			
Nombre chenilles	14	5	1
Poids moyen	12	37	70
Activité/larve	1,3	3,7	3
" corrigée	4	11,47	-
Activitée/mmg	0,11	0,07	-
" corrigée	0,34	0,22	-
4e lot (8.10)			
Nombre chenilles	9	5	13
Poids moyen	11,4	47,2	86
Activité/larve	4,5	27,4	11,2
" corrigée	9	55	22,4
Activité/mmg	0,4	0,58	0,14
" corrigée	0,8	1,16	0,28

Discussion

Le nombre de chenilles ramassées et leur répartition en catégorie sont très variables, suivant l'évolution de l'espèce en plein champ. Les nombres obtenus par l'exploitation de ce tableau ne peuvent pas être interprétés dans un sens strict.

Cependant, on notera que l'activité par larve semble maximale pour la catégorie moyenne.

Si l'on se rapporte à l'activité au mmg de matière sèche, on constate que ce sont les larves les plus jeunes qui présentent généralement le maximum: elles sont dans une phase active de croissance et assimilent rapidement le phosphore, tandisque les larves dans leur phase finale accumulent surtout des lipides peu riches en phosphore.

En fonction de l'éloignement du traitement, on peut constater que le dernier lot donne encore des chiffres très nets permettant une distinction moyenne sans ambiguïté. Il est probable que le temps moyen d'alimentation sur les cotonniers marqués a été plus long que pour le lot n° 3, d'où souvent des chiffres plus favorables que pour celui-ci.

Dans l'expérience finale projetée, où les larves se nourriront toute leur vie sur cotonniers marqués, on peut donc espérer un diagnostic valable trois à quatre mois après, dans le cas du ^{32}P.

Quant aux chenilles n'ayant montré aucune activité, leur nombre diminue avec leur âge, et il reste des chenilles âgées non marquées simplement parce qu'elles ont été placées sur la parcelle peu de temps avant le prélèvement (tableau II).

TABLEAU II

CHENILLES N'AYANT MONTRÉ AUCUNE ACTIVITÉ

	0 - 30	30 - 60	> 60
Nombre chenilles	13	2	3
% total	30%	11%	11%

Résultats avec d'autres chenilles

En même temps que Diparopsis - mais en nombre plus limité puisque ce n'était pas l'objet principal de l'essai - on a récolté des insectes variés : Sauterelles, Dysdercus etc. Ce sont les chenilles de Prodenia, de Sylepta et de Cosmophila et Heliothis qui ont donné lieu à la remarque générale suivante :

Une bonne partie des échantillons d'Heliothis montraient une activité en gros comparable à la catégorie de Diparopsis correspondante. Mais les chenilles strictement phyllophages (Cosmophila et Sylepta) n'ont montré aucune activité.

Aussi donc, l'emploi du ^{32}P pour ce genre de travaux conduit à une limitation dont il faut rechercher la cause dans le métabolisme même de

cet élément dans la plante. En effet, bien que l'absorption ait eu lieu pour une large part directement à travers le limbe foliaire, une partie de la pulvérisation ayant atteint le sol, le transport du phosphore vers les extrémités en croissance active et surtout vers les méristemes « nobles » devant donner des organes florifères se fait très rapidement. Les chenilles devorant seulement le limbe des feuilles ne sont donc pas marquées, tandis que Heliothis et Diparopsis qui se nourrissent presque exclusivement de boutons floraux, de fleurs et de jeunes capsules, sont le plus souvent bien marqués.

Il ne faut donc pas s'attendre, avec cette méthode de pulvérisation directe, à des résultats positifs pour n'importe quel insecte.

DEUXIÈME ESSAI PRÉLIMINAIRE

Nous avions donc vérifié l'existence d'une possibilité d'utilisation du ^{32}P en certaines conditions : saison des pluies, croissance encore active du cotonnier, etc. Après cet encouragement, il importait de se placer dans un cadre à la fois élargi et plus proche des conditions normales des recherches biologiques qui constituent le but réel.

La saison chaude s'est prolongée anormalement en 1962, et ce n'est qu'à la fin novembre que les températures nocturnes s'abaissèrent en dessous de 16°.

Le 4 décembre on procéda à une deuxième application dans des conditions voisines de la première, mais comportant cette fois du ^{32}P et du ^{35}S.

L'application eut lieu par temps sec et calme, le matin sur des cotonniers âgés mais ayant reçu des irrigations afin de susciter un nouveau départ de végétation en contresaison.

A l'expédition le 13 novembre les activités totales étaient 16 mc pour le ^{32}P et 2,7 cm^3 pour le ^{35}S. Au moment de la pulvérisation, il restait donc respectivement 6 mc et 1,7 cm^3.

La plus longue période du ^{35}S permettra peut-être d'allonger la durée et la précision des observations. Des expériences classiques ont en effet montré que le soufre joue un rôle capital dans l'alimentation du cotonnier, qui en réclame des quantités du même ordre de grandeur que celles de phosphore et d'azote. Il reste à savoir si la migration du soufre à partir de la feuille se fait de façon différente de celle du phosphore et si le métabolisme du soufre chez les insectes variés permettra le repérage par ce traceur.

Les observations se poursuivent actuellement, et les résultats ne sont pas encore connus pour l'instant.

DISCUSSION

C. H. SCHMIDT: In Table I it is not clear to me what units you are using in relation to the activity of the larvae. Would you elucidate?

R. DELATTRE: The figures given in Table I are the average figures for total counts per five minutes in an ordinary G-M counter. We do not wish to attach any precise quantitative meaning to these figures, but simply to show that the positive or negative response provides reasonable certainty, despite the fact that counting is carried out away from the actual site of the experiment.

USE OF ISOTOPES FOR INVESTIGATING THE BEHAVIOUR AND ECOLOGY OF INSECT PESTS IN SOME RECENT STUDIES

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Abstract — Résumé — Аннотация — Resumen

USE OF ISOTOPES FOR INVESTIGATING THE BEHAVIOUR AND ECOLOGY OF INSECT PESTS IN SOME RECENT STUDIES. Investigations into the ecology, behaviour, dispersal and longevity of insects have always provided challenges to the entomologist. The use of isotopes is an effective tool and the following is a report on some interesting problems solved by their application.

Anopheles stephensi is the main vector of malaria in southern Iran. On P^{32}-labelled mosquitoes, the dispersal, behaviour, digestion of blood meal, maturation of ovaries and length of gonotrophic cycles were successfully worked out. It was found that in about 80% of the cases the mosquito needed two blood meals for the completion of the first cycle. The first cycle itself was completed in 4-5 d depending upon the temperature.

Labelled mosquitoes which had emerged overnight were released in an isolated village. The ratio of active mosquitoes to total catch was worked out every day and thus, on the assumption that the natural population remained constant, the mortality rate, which was found to be exponential for the first six days, was worked out.

The mating behaviour of the female was also studied by using normal females which had mated once with P^{32}-labelled males. It was found that the female mates more than once and that after mating with an active male, the spermatheca became active. Counts of up to twice the background (12 counts/min) were obtained by using males giving about 15 000 counts/min.

Studies of the injection of saliva in glucose solution during feeding were also made on P^{32}-labelled mosquitoes.

Eurygaster integriceps is a serious pest of wheat in Iran, Pakistan and the Middle East. Using P^{32}-activated wheat plants, the feeding behaviour of the first-instar nymph was investigated. Other foods, radioactively labelled, were also studied and it was found that feeding was essential for the first moult, even if the food consisted of water absorbed in a filter paper.

EMPLOI DES RADIOISOTOPES DANS DES RECHERCHES RÉCENTES SUR LE COMPORTEMENT ET L'ÉCOLOGIE DES INSECTES NUISIBLES. Les recherches sur l'écologie, le comportement, la dispersion et la longévité des insectes ont toujours posé des problèmes intéressants aux entomologistes. Les radioisotopes constituent un outil efficace, et l'auteur expose les résultats auxquels il est parvenu grâce à leur emploi dans l'étude de certains de ces problèmes.

L'Anopheles stephensi est le principal vecteur du paludisme en Iran méridional. En marquant des moustiques avec ^{32}P, on a pu déterminer la dispersion, le comportement, la digestion du sang, le développement des ovaires et la durée des cycles gonotrophiques. On a constaté que, dans environ 80% des cas, le moustique a besoin de deux repas de sang pour réaliser le premier cycle. La durée de ce cycle a été de 4-5 j selon la température.

Les moustiques marqués qui étaient parvenus à l'état adulte pendant la nuit ont été lâchés sur un village isolé. On a calculé tous les jours le pourcentage de moustiques irradiés par rapport à la prise totale; en partant de l'hypothèse que la population naturelle demeurait constante, on a déterminé le taux de mortalité et constaté que c'était une fonction exponentielle pour les six premiers jours.

On a également étudié le comportement sexuel de la femelle à l'aide de femelles normales qui s'étaient accouplées une fois avec des mâles marqués avec ^{32}P. On a pu établir que la femelle s'accouple plus d'une fois et que la spermatothèque devient radioactive après accouplement avec un mâle irradié. On a enregistré un nombre de coups pouvant atteindre jusqu'à deux fois le bruit de fond (12 cpm) en utilisant des mâles donnant environ 15 000 cpm.

Avec des moustiques marqués avec ^{32}P, on a également étudié l'injection de salive dans une solution de glucose pendant l'absorption de nourriture.

L'Eurygaster integriceps est un insecte très nuisible au blé en Iran, au Pakistan et au Moyen-Orient. Au moyen de grains marqués par ^{32}P, on a étudié le comportement alimentaire au premier stade nymphal. On a également étudié d'autres nourritures marquées et on a constaté que la nymphe devait se nourrir, ne serait-ce que d'eau absorbée par un filtre en papier, pour franchir le premier stade de son évolution.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ ДЛЯ ИССЛЕДОВАНИЯ ПОВЕДЕНИЯ И ЭКОЛОГИИ НАСЕКОМЫХ-ВРЕДИТЕЛЕЙ В ИРАНЕ. Исследования экологии, поведения, распространения и срока жизни насекомых всегда привлекали энтомологов. Использование радиоизотопов является эффективным орудием исследования; ниже дается сообщение относительно нескольких интересных проблем, разрешенных с их помощью.

Anopheles Stephensi является главным переносчиком малярии в южном Иране.

Благодаря использованию меченных P^{32} комаров были получены сведения относительно их распространения, поведения, усвоения ими крови, созревания яичников и продолжительности гонотрофических циклов. Было установлено, что преимущественно в 80% случаев для завершения первого цикла комарам требовалось дважды кровяное питание. Сам первый цикл завершался в зависимости от температуры за четыре или пять суток.

Выплодившиеся за ночь меченые комары выпускались на свободу в изолированной деревне. Соотношение между активными комарами и общим их уловом устанавливалось каждый день и, при условии неизменности природной популяции, определялась смертность, причем оказалось, что она в течение первых шести суток являлась экспоненетной.

Было изучено также брачное поведение самок путем использования нормальных самок, спаривавшихся в прошлом один раз с меченными P^{32} самцами. Было установлено, что самка спаривается более одного раза и что после спаривания с активным самцом сперматека становится активной. Используя самцов, дававших 15 000 отсчетов в минуту, были получены счеты, вдвое превышавшие фоновые (12 счетов в минуту).

При помощи использования меченных P^{32} комаров были проведены также исследования по инъекции слюны в раствор глюкозы во время питания.

Eurygaster intergriceps является опасным вредителем пшеницы в Иране, Пакистане и на Среднем Востоке.

С помощью пшеницы, меченной P^{32}, был изучен режим питания куколки на ее первовозрастной стадии. Были также изучены другие виды пищи, меченной радиоактивными веществами, и обнаружено, что питание необходимо в период первой линьки, если даже пища состоит из воды, впитанной фильтровальной бумагой.

EMPLEO DE ISÓTOPOS EN ESTUDIOS RECIENTES SOBRE EL COMPORTAMIENTO Y LA ECOLOGÍA DE LAS PLAGAS INSECTILES. Las investigaciones sobre la ecología, el comportamiento, la distribución y la longevidad de los insectos suelen ofrecer a los entomólogos problemas de difícil solución. Los isótopos han resultado auxiliares muy eficaces para estas investigaciones; en la memoria se informa sobre algunos problemas interesantes que se han podido resolver gracias a su empleo.

El Anopheles stephensi es el principal vector palúdico en el sur del Irán. Empleando mosquitos marcados con ^{32}P se pudo determinar su distribución, su comportamiento, la digestión de la sangre, la maduración de los ovarios y la longitud de los ciclos gonotróficos. Se comprobó que en un 80 por ciento de los casos el mosquito necesita alimentarse dos veces con sangre para completar el primer ciclo, cuya duración es de 4 a 5 días según la temperatura.

A los mosquitos marcados que surgían de la ninfa durante la noche se los liberaba en una aldea aislada. Diariamente se calculaba el porcentaje de anofeles capturados que estaban marcados y así, suponiendo que el número de mosquitos de la zona permanencía constante, se pudo determinar el índice de mortalidad, que resultó ser exponencial durante los seis primeros días.

También se estudió la copulación de las hembras utilizando hembras normales que habían copulado una vez con machos marcados con ^{32}P. Se pudo observar que las hembras copulan más de una vez y después de hacerlo con un macho activado sus espermatotecas se hacían también radiactivas. Con machos que daban hasta 15 000 impulsos/min se obtuvieron cifras de recuento de hasta el doble del fondo parasitario (12 impulsos/min).

Con mosquitos marcados con ^{32}P se hicieron estudios sobre la inyección de saliva en una solución de glucosa durante el proceso de alimentación.

El Eurygaster integriceps es una plaga muy peligrosa del trigo en el Irán, el Pakistán y el Oriente Medio. El autor investigó el modo de alimentarse de las ninfas del primer estadio utilizando plantas de trigo activadas con ^{32}P. También se estudiaron otros alimentos marcados radiactivamente y se encontró que la alimentación es esencial para la primera muda, aunque consiste solamente en agua embebida en un papel de filtro.

INTRODUCTION

Investigations into the ecology, behaviour, dispersal and longevity of insects have always provided challenges to the scientist. From the early days the research worker has used a variety of ingenious aids in the pursuit of his knowledge, but more often than not his attempts have been thwarted because no matter how intricate the aids used, they failed to match the complexities of the situation involved.

Studies of the movements of small larvae underground, or the frequency of the mating of a eurygamous species in nature, or the food and feeding habits of a predator, apparently seem to be simple, but the solutions of problems of that kind have defied the ingenuity of the scientist for a long time. Only since isotopes were made available has the research worker found a potent weapon of attack.

In this paper I intend to give an account of the use of isotopes in the solution of a few intricate problems related to pests of agricultural and public health importance. In fact, the health of the rural population is directly related to agricultural production, and control of a disease like malaria has been shown by the World Health Organization team working in Pakistan to result in a substantial increase in food production [1].

USE OF P^{32} IN THE STUDY OF THE BIOLOGY OF ANOPHELES STEPHENSI

Malaria has since time immemorial plagued the tropical countries, sapping the energy of the inhabitants, reducing their productivity and seriously affecting food production. The high hopes for malaria eradication placed in residual insecticides were soon belied by the discovery of resistance in vector mosquitoes. This discovery has once again emphasized the value of the study of the biology and ecology of insects affecting the economy of a country.

With the aid of P^{32} we have successfully studied the following aspects of the biology of Anopheles stephensi:

(i) Dispersal;
(ii) Digestion of blood meal, simultaneous development of ovaries and length of gonotrophic cycle in nature;
(iii) Mortality rate; and
(iv) Mating behaviour.

This mosquito is one of the most serious vectors of malaria in the region and has developed a very high degree of resistance to the insecticides used for malaria eradication.

Mosquitoes were labelled by rearing IVth-instar larvae in water containing 10 μc of P^{32} per litre per 500 larvae. Radioactivity measurements of about 4000 counts/min in the case of males and about 5000 counts/min

in the case of females, as detected in the top shelf of an Ericsson scaling unit type 1221C, were obtained [2].

Several thousands of mosquitoes which had emerged overnight were released at a predetermined point in the evening. Starting next morning, regular collections were made in neighbouring villages. All collections were monitored to study the dispersal of the labelled mosquitoes. Radioactive A. stephensi were captured up to 4.5 km from the point of release [3].

All radioactive females were dissected to study the digestion of the blood meal (Sella stage) and the development of ovaries (Christopher's stage). Our studies showed that in the majority of cases the first cycle was completed within five days (about 110 h) after release when the mean daily temperature during the period of trial varied between 28.3°C and 29.6°C and the mean relative humidity between 21.5% and 33.2% [4].

Radioactive A. stephensi which had emerged overnight were released in isolated villages and the ratio of radioactive mosquitoes to the total catch was worked out daily. On the assumption that the natural population was constant, the daily fall in this ratio gave us an idea of the daily mortality of the labelled mosquitoes. This mortality rate was found to be exponential [3].

Since the successful eradication of screw-worm fly, the study of the mating behaviour of insects has assumed importance. Using radioactive male A. stephensi we studied the mating behaviour of this species. When normal females were mated with radioactive males (7000 - 13 000 counts/min) detectable radioactivity was acquired by the spermathecae (17.5 - 25 counts/min). The background in this case was 12 counts/min. It was thus proved that the female of A. stephensi mates more than once. (QURAISHI and ARTHUR [5]).

Before releasing labelled mosquitoes we wanted to find out the amount of radioactivity a female would transfer to an animal being bitten. Even when several hundred radioactive females (about 5000 counts/min) were allowed to feed on a guinea-pig there was no detectable radioactivity transferred to its blood. We therefore tried to find out the radioactivity transferred to sugar solution when radioactive A. stephensi fed on it. Diluted honey absorbed in a thin layer of cotton was counted for its radioactivity and kept overnight in a cage of freshly emerged mosquitoes. Next morning the radioactivity of the honey solution was determined and the mosquitoes which had fed on it were collected and their radioactivity was determined separately. Several experiments thus conducted showed that while feeding the mosquitoes transferred about 0.1% of their total radioactivity to the honey on which they fed. (QURAISHI, unpublished data.)

USE OF ISOTOPES IN THE STUDY OF THE ECOLOGY OF EURYGASTER INTEGRICEPS

Eurygaster integriceps Put., known locally as Senn pest, is a serious pest of wheat and to a lesser extent of barley in the Middle East, Turkey and Southern USSR. Though some literature is available on the feeding habits and behaviour of the various stages of this insect, descriptions are sometimes conflicting and at times lack conclusive evidence. FEDOTOV [6]

states that the main reserves of food are built up in larval stages, whereas VODJDANI [7] is of the opinion that in the first instar the insect does not feed. This interested us in the feeding habits of the first-instar nymph. Wheat plants were made radioactive by growing them in Hoagland's water culture medium containing P^{32}. Freshly emerged first-instar nymphs were then released on the plants in specially prepared plastic containers. After 24 h the nymphs were removed, cleaned by a brush dipped in phosphorus carrier to remove any external contamination, and counted. Considerable radioactivity was recorded in the nymphs, proving conclusively that they did feed on the plants. P^{32}-activated sugar solutions and water of known activity were absorbed in filter papers and tried as an alternative food for first-instar nymphs of known weight. It was found that the radioactivity of the fed nymph was nearly equal to the radioactivity of the weight of liquid imbibed [8].

By using a label of tantalum-182 $0.05 \times 0.16 \times 0.46$ mm in size (specific activity 8 μc) glued to the scutellum of the insect, the horizontal and vertical movements and daily feeding habits of six young adults were studied in open (42 wheat stems per 0.25 m^2) and closed (82 wheat stems per 0.25 m^2) wheat fields in Northern Iran, for three to four days at the beginning and end of the adult feeding period.

It was found that on an average the labelled insects changed their position four times a day and moved about 60 - 70 cm each day. Those in the open field moved more often and covered somewhat longer distances.

The labelled insects in closed wheat spent nearly the whole time above ground but the bugs in the open wheat spent a considerable time in cracks in the soil.

Feeding occurred chiefly in daytime. It increased in frequency from sunrise to a maximum at 8 a.m., declined to a minimum at midday and rose to another peak in the afternoon. Feeding almost ceased at sunset.

At the end of the feeding period, just before their flight to the mountains, the insects spent most of the time in cracks in the soil and were rarely seen feeding [9].

Microphanurus sp. is being reared on a large scale for the biological control of Senn pest in Iran and in order to plan effective releases of this parasite for that purpose it is important to find out its effective flight range. Attempts were made to label the parasite by rearing it in radioactive eggs of E. integriceps. To obtain radioactive eggs, female Senn pest were either fed on wheat soaked in P^{32} or injected with 10 μc of P^{32} isotonic solution. An activity of up to 100 counts/min was measured in the eggs and parasites reared in them became radioactive. However, the activity was not sufficient for our purpose and we are trying other methods of obtaining radioactive parasites [8].

I have in this short paper endeavoured to discuss in broad outline the uses to which we have put isotopes in our studies. Most of this work would have been extremely difficult to carry out without their help; besides, the use of other labels like dusts, powders or paints would have involved unnecessary handling or anaesthetizing of the insects, thereby adding a factor of uncertainty to the validity of our results.

ACKNOWLEDGEMENTS

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DISCUSSION

W. KLOFT: Some of your results are very interesting for me, such as, for example, the ejection of radioactive saliva by mosquitoes. We did similar work with plant-sucking insects, but I think this is the first time that the ejection of labelled saliva has been demonstrated in a blood-sucking insect.

You have also tested how often the Anopheles female mates during her life, by making the sexual organs radioactive. From experience with transfer of labelled substances in the case of social Hymenoptera, we have found that after mating by the male, sometimes only the radioactive secretion of the accessory glands has been transferred. The sperm is not always transferred but only the accompanying radioactive secretion. You have referred to the amount of mating, not of transference, I believe.

M.S. QURAISHI: That is very interesting, Dr. Kloft. As regards the mating habits of the Anopheles female, we only studied the activity of the spermatheca of the female. Subsequently these results were confirmed by field observations where we collected copulating females from swarms and dissected them. We were able to find females which showed one dilatation and two dilatations in their ovarioles, thereby proving conclusively that females which had laid one or two batches of eggs were coming again to mate in these swarms.

It would be very difficult to prove the transfer of sperms conclusively in subsequent matings, because the spermatheca would already have a store of spermatozoa from previous mating. Autoradiography may help.

C.H. SCHMIDT: I was interested in what you said in your oral presentation with regard to honey solution and hot mosquitoes. Is there any possibility that instead of being just regurgitations, you could have defecation by the mosquitoes? We have done some work with Aëdes aegypti and the cages after a while get highly contaminated through the hot excretion.

M.S. QURAISHI: Actually these mosquitoes were captured the very next day. They were only kept overnight with the solution of honey, so I do not think there would be very much contamination due to excretion. Also, when female mosquitoes feed on honey solution you can easily see the distended abdomen, with the solution inside.

C.H. SCHMIDT: You showed that they went about 4.5 km. Was there any prevailing wind during your experiment? Or was there no wind?

M.S. QURAISHI: We kept a good record of wind velocity. It was usually between 2 and 5 knots. Of course there were one or two cases when it was higher than that. Though it is not statistically significant, the first catches of mosquitoes, which flew overnight, were found in a direction almost opposite to the direction of the wind.

C.C. HASSETT: May I say in relation to wind transport of mosquitoes, that I found in field experiments in Canada that A. aegypti were not affected by wind because at wind velocities of over 5 mile/h the insects took cover in vegetation.

ESSAI PRÉLIMINAIRE AVEC ^{32}P SUR LA DISPERSION DES ADULTES DU DACUS OLEAE GMEL

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Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDY WITH P^{32} ON THE DISPERSION OF ADULTS OF THE OLIVE FLY (DACUS OLEAE GMEL). The lack of numerical data on the movement of the olive fly led to a preliminary trial with 2500 adults reared in cages and labelled before release with a sugar solution containing 20 μc P^{32}/cm^3.

Subsequent measurements showed dispersion of the labelled adults up to a distance of 2 km from the point of release during a calm sunny period in winter.

ESSAI PRÉLIMINAIRE AVEC ^{32}P SUR LA DISPERSION DES ADULTES DU DACUS OLEAE GMEL. Le manque de données numériques sur la dispersion de la mouche de l'olive a amené à faire un essai préliminaire avec 2500 adultes élevés dans des cages et marqués, avant leur lâcher, au moyen d'une solution sucrée contenant 20 μc de ^{32}P par centimètre cube

Les mesures ultérieures ont montré une dispersion des adultes marqués jusqu'à une distance de 2 km du point de lâcher pendant les jours ensoleillés d'hiver, dits alcyoniens.

ПРЕДВАРИТЕЛЬНЫЕ ОПЫТЫ С P^{32} ДЛЯ ИЗУЧЕНИЯ РАССЕЯНИЯ ВЗРОСЛЫХ ОСОБЕЙ (DACUS OLEAE GMEL). Отсутствие количественных данных относительно расселения оливковых мух обусловило проведение предварительного опыта с 2500 взрослыми особями, выращенными в ящиках и помеченными перед выпуском с помощью сладкого раствора, содержащего 20 мкк на 1 $см^3$ P^{32}.

Относительные измерения показали удаление меченых взрослых особей до 2 км от места выпуска в период солнечных зимних дней, называемых алкионидными.

ESTUDIO PRELIMINAR CON ^{32}P DE LA DISPERSIÓN DE LOS ADULTOS DEL DACUS OLEAE GMEL. La falta de datos numéricos sobre la dispersión de la mosca del olivo ha inducido a efectuar un estudio preliminar con 2 500 adultos criados en cajas y marcados antes de su suelta con una solución azucarada que contenía 20 μc de $^{32}P/cm^3$.

Las mediciones han puesto de manifiesto una dispersión de los adultos marcados hasta una distancia de dos kilómetros del punto en que fueron soltados durante los días soleados de invierno llamados alcióneos.

Diverses opinions ont été formulées depuis longtemps concernant la dispersion et l'immigration des adultes de la mouche de l'olive. Les essais que nous avons faits pendant les années 1957–1961 avec divers produits attractifs, c'est-à-dire avec des hydrolysats de protéines, ont démontré que le tropisme des adultes du Dacus d'un point donné vers un autre dépend considérablement de la différence de l'humidité relative qui existe entre ces deux points [2].

Ces résultats, qui étaient d'ailleurs en accord avec les données d'ACREE et al. [1] sur la Musca domestica, montraient qu'il était probablement possible d'avoir un mouvement d'adultes des endroits à faible humidité relative vers des points ayant une humidité relative élevée. Ces observations étaient, en plus, en conformité avec l'opinion répandue parmi les agronomes et oliviculteurs de notre pays, selon laquelle la mouche de l'olive attaque précocement les arbres situés dans des endroits humides, d'où la notion des «foyers» du Dacus. La manque des données nu-

mériques sur la dispersion de la mouche de l'olive nous a amenés à exécuter un essai péliminaire avec des mouches de l'olive marquées au ^{32}P élevées dans des cages, d'après la méthode appliquée par SACANTANIS [3], et provenant des olives infestées.

Les olives infestées restaient pendant 25 à 30 j approximativement sur des tamis permettant le passage des larves et chaque jour on collectait les larves du troisième stade ainsi que les pupes produites.

Les adultes apparus ensuite, après l'éclosion des pupes, étaient introduits dans des cages d'insectes et étaient nourris d'une part avec des morceaux de fruits (figues, bananes) et d'autre part avec une solution aqueuse contenant une partie de levure de bière, une partie de protéine hydrolysée partiellement – de la maison «Nutritional Biochemicals» des Etats Unis – et huit parties de sucre.

Les adultes restaient ensuite à jeun et sans eau pendant 24 h; 24 h avant leur lâcher on leur donnait une solution sucrée contenant 20 $\mu c/cm^3$ de phosphore marqué sous la forme d'acide phosphorique (neutralisé au moyen de $NaHCO_3$) et provenant d'un produit concentré (10 mc/ml) de l'«Atomic Energy of Canada Limited».

La détermination de la concentration en ^{32}P était basée d'une part sur les observations de VIEL et CHANCOGNE [4], d'après lesquelles les adultes d'une espèce voisine, les Ceratites, pouvaient absorber per os une quantité de 1 mm^3 d'eau, et d'autre part sur les données de YATES et LINDQUIST [5] d'après lesquelles une quantité de 0,02 μc était suffisante pour le marquage des espèces Musca domestica et Phormia regina.

La solution sucrée à ^{32}P était mise dans des flacons suspendus sur le plafond des cages et contenant du coton en forme cylindrique, dont l'un des bouts se trouvait au fond du flacon et l'autre sortait du goulot.

La manque d'infestation précoce dans la région des expériences ne nous a pas permis d'avoir un nombre suffisant de mouches pendant l'automne. C'est pourquoi le lâcher des adultes a été fait pendant les jours ensoleillés de janvier dits «Alcyonides».

Nous avons utilisé environ 2500 mouches de l'olive ainsi que 62 pièges contenant 300 cm^3 d'une solution 2% de sulfate d'ammonium. Les pièges étaient suspendus sur des branches des arbres de l'oliveraie et placés sur les circonférences de cercles ou arcs de cercles dont les rayons étaient de 100, 400, 1000 et 2000 m approximativement. La distance entre les pièges sur les circonférences ou les arcs était d'environ entre 40 et 150 m.

L'étendue relativement faible de la surface de l'oliveraie expérimentale dans laquelle nos essais sur la lutte de la mouche de l'olive ont été exécutés pendant 1961 ne nous a pas permis de mettre des pièges à une distance supérieure à deux kilomètres. Mais nous avions l'opinion que même cette distance pouvait donner une image sur la dispersion de la mouche de l'olive, dispersion au sujet de laquelle rien n'était établi positivement jusqu'à cette année.

On peut voir des détails sur la disposition des pièges dans l'oliveraie expérimentale dans le diagramme schématique de la figure 1. Le dénombrement des mouches capturées dans des pièges a été fait quatre fois, pendant une période de neuf jours, après le lâcher des adultes marqués.

L'estimation de l'irradiation des mouches a été faite à l'aide d'un appareil G-M (type N. 529 C. Scaler) de la maison «ECKO Electronics Ltd.»,

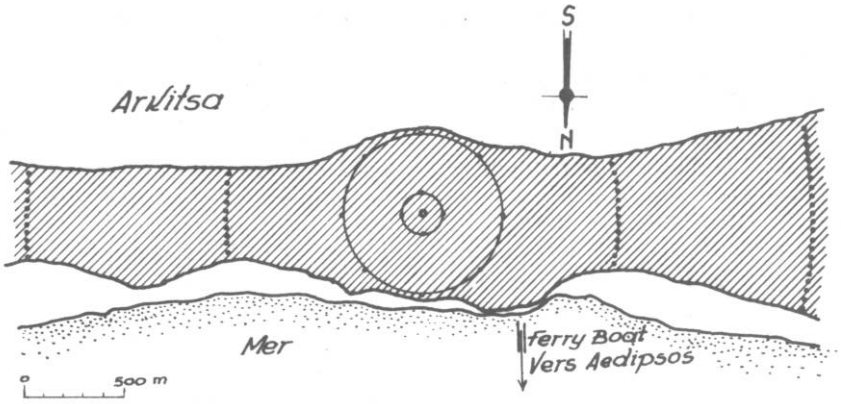

Figure 1

Disposition des pièges dans l'oliveraie expérimentale
- Pièges
- O Point de lâcher des adultes marqués

Angleterre. Ces mesures ont mis en évidence une dispersion des adultes marqués dans les oliveraies jusqu'à une distance de deux kilomètres, au moins, du point de lâcher pendant les jours ensoleillés de janvier dits « Alcyonides ».

Les adultes marqués présentaient une irradiation jusqu'à 1350 cpm; leur activité ne surpassait donc pas celle observée par YATES et LINDQUIST [5] pendant les essais sur Musca domestica et Phormia regina.

Il faudrait signaler à titre de comparaison que le bruit de fond était en même temps de 9 à 15 cpm approximativement.

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DISCUSSION

J. LECOMTE: What proportion of tagged flies did you capture out of the 2500 released?

P.S. ORPHANIDIS: We trapped about 1.2% of the adults released.

J. LECOMTE: After measuring the activity of the captured flies, did you release them or destroy them?

P.S. ORPHANIDIS: After measurement of their activity the flies were kept in the laboratory.

J. LECOMTE: The number of tagged flies was thus decreasing with time?

P.S. ORPHANIDIS: Yes.

C.H. SCHMIDT: What was the total number of flies, both tagged and non-tagged, recovered in your traps?

P.S. ORPHANIDIS: We captured 1950 flies.

M. FERON: What was the lowest activity measured amongst the captured flies?

P.S. ORPHANIDIS: The minimum was 3 to 4 times the background.

G. SILVA: Could you tell us something about the temperature in January? It is quite remarkable that you found flies kilometres away in that month.

P.S. ORPHANIDIS: Naturally, during the cold months there cannot be a satisfactory dispersal of insects. Our intention was to release the flies during the autumn months, but owing to the late infestation the number of infested olives was not enough to produce a sufficiently high number of adult flies. If the tests had been carried out in the autumn I think a much greater dispersal would have been found. In Greece, however, there is a period of approximately 15 d in January during which the temperature is relatively high, lying between 12 and 16°C during the day and 7 and 10°C at night. Release and capture took place during this period.

C.T. LEWIS: I have been concerned with some work on the migration of Orthotylus virescens bugs in broom plantations. We found quite an interesting phenomenon when we released bugs in the centre of the plantation, marked with P^{32} and others along the boundary of the plantation marked with S^{35}. The bugs released at the centre did not move about very much, but the bugs that were released at the edge of the plantation migrated quite freely. If we had marked all the bugs with the same isotope, we would have got a very small percentage migrating, but in fact, as I say, along the boundary quite a high percentage of bugs migrated and this quite altered the picture we had of the movements of the insects. I wonder whether this difference in behaviour between the centre of the plantation and the boundary might also exist with Dacus oleae.

P.S. ORPHANIDIS: In our case the labelled adults were all released at a point in the centre of the olive grove.

M.S. QURAISHI: I have three question. How soon after emergence does mating take place in the case of Dacus oleae? How soon after mating are eggs laid? And is there any difference in the flight range before mating and after mating?

P.S. ORPHANIDIS: This was just a preliminary study and we did not intend to investigate anything but the dispersal of a given insect population in relation to the age of the insects. It appears, however, from our observations, that egg-laying takes place 8 — 12 d after emergence.

PRELIMINARY STUDIES OF THE FIELD MOVEMENT OF THE OLIVE FRUIT FLY (DACUS OLEAE GMEL.) BY LABELLING A NATURAL POPULATION WITH RADIOACTIVE PHOSPHORUS (P^{32})

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Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDIES OF THE FIELD MOVEMENT OF THE OLIVE FRUIT FLY (DACUS OLEAE GMEL.) BY LABELLING A NATURAL POPULATION WITH RADIOACTIVE PHOSPHORUS (P^{32}). Preliminary trials were conducted to obtain the first data on the field movement of the olive fruit fly, Dacus oleae Gmel. These studies were carried out in the olive-growing area at Rovies, Evvia, Greece, during the autumn of 1961, when the maximum adult fly activity usually occurs.

A mixture of radioactive P^{32} of $H_3P^{32}O_4$ in HCl and hydrolysate protein, Staley No. 7, was prepared for labelling the naturally occurring adult fly population. Twigs of 30 olive trees which were selected in a semi-mountainous olive orchard at Rovies were treated with the P^{32} bait solution on 14 October 1961.

Five hundred McPhail traps, containing 3% of diammonium phosphate as a lure, were used for the collection of flies. Traps were distributed in the olive groves of the entire area at Rovies and also in the adjacent areas (forest or olive groves) up to a distance of 10 km from the treated trees.

A total of 350 tagged flies of both sexes were collected in 48 traps during 15 counts of traps made between 15 October and 18 November. Labelled flies which were caught from 1 to 35 days after treatment represented 2.8% of the total number of flies collected in 48 traps, or 0.15% of the total number of flies caught in 298 traps in the entire area at Rovies. A proportion of 73% of the tagged flies were trapped within the first ten-day period following treatment. Labelled flies were found as far as 4300 m (maximum of dispersal) from the labelling station. Radioactivity of tagged flies ranged between 258 and 9549 counts/min per fly (background radiation 8-21 counts/min).

These preliminary trials have confirmed a more or less continuous local movement (dispersal) of flies from the semi-mountainous grove to the adjacent plains or to the coastal olive groves at Rovies from the north to south. Long-distance movement (migration) of Dacus flies to other areas has not been observed. Pine woods appear to act as a barrier to the movement of Dacus oleae adults.

ÉTUDES PRÉLIMINAIRES SUR LES DÉPLACEMENTS DE LA MOUCHE DE L'OLIVE (DACUS OLEAE GMEL.) PAR MARQUAGE D'UNE POPULATION NATURELLE AU RADIOPHOSPHORE (^{32}P). Les auteurs ont procédé à des expériences préliminaires pour recueillir des données de base sur les déplacements de la mouche de l'olive (Dacus oleae Gmel.). Ces études ont été menées dans les oliveraies de Rovies, Evvia, en Grèce, à l'automne 1961, à l'époque où les mouches adultes sont particulièrement actives.

Les auteurs ont préparé un mélange de H_3-^{32}P-O_4 dans HCl et d'hydrolysat de protéine, Staley n° 7, pour marquer une population naturelle de mouches adultes. Une nourriture alléchante à base de la solution marquée à été appliquée le 14 octobre 1961 sur les branches de 30 oliviers choisis dans une oliveraie semi-montagneuse, à Rovies.

Les mouches ont été capturées à l'aide de 500 pièges McPhail, contenant 3% de phosphate diammonique qui servait d'appât. Les pièges avaient été répartis dans les oliveraies de Rovies ainsi que dans les zones adjacentes (forêts ou oliveraies) dans un rayon de 10 km autour des arbres traités.

Au total, 350 mouches marquées des deux sexes ont été recueillies dans 48 pièges au cours de 15 relevés effectués entre le 15 octobre et le 18 novembre. Les mouches marquées, qui ont été capturées de 1 à 35 jours après le traitement, représentaient 2,8% du nombre total des mouches recueillies dans les 48 pièges ou 0,15% du nombre total des mouches capturées dans 298 pièges dans toute la zone de Rovies; 73% des mouches marquées ont été capturées dans les 10 premiers jours après le traitement. On a trouvé des mouches marquées jusqu'à 4300 m des arbres portant la solution marquée (maximum de dispersion). La radioactivité des mouches marquées était comprise entre 258 et 9549 cpm par mouche (rayonnement ambiant, 8 à 21 cpm).

Ces expériences préliminaires ont confirmé l'existence d'un déplacement local plus ou moins continu (dispersion) des mouches entre l'oliveraie semi-montagneuse et les plaines adjacentes ou les oliveraies côtières de Rovies, suivant une direction nord-sud. On n'a pas observé de déplacement à grande distance (migration) des mouches vers d'autres régions. Il semble que les bois de pins opposent un barrage aux déplacements des adultes de la Dacus oleae.

ПРЕДВАРИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ПОЛЕВОГО ПЕРЕМЕЩЕНИЯ ОЛИВКОВОЙ ФРУКТОВОЙ МУХИ (DACUS OLEAE GMEL.) ПОСРЕДСТВОМ МЕЧЕНИЯ ЕСТЕСТВЕННОЙ ПОПУЛЯЦИИ РАДИОАКТИВНЫМ ФОСФОРОМ (P^{32}). Проведены предварительные эксперименты для получения первых данных о полевом перемещении оливковой фруктовой мухи Dacus oleae (Gmel.). Эксперименты были проведены в районе произрастания олив в Ровиес, Эвиа, Греция, в течение осени 1961 года, когда обычно наблюдается максимальная активность взрослых особей мухи.

Для мечения естественных популяций взрослых особей был приготовлен состав Стэли № 7, состоящий из смеси P^{32} в виде $H_3P^{32}O_4$ в соляной кислоте и в гидролизате белка. 14 октября 1961 года ветви 30 оливковых деревьев, выбранных на полугорной оливковой плантации в Ровиес, были обработаны раствором приманки, меченной P^{32}.

Для отлова мух использовались 500 ловушек Мэкфейла с 3% диаммонийфосфата в качестве приманки. Ловушки были расставлены в оливковых рощах всего района Ровиес, а также в соседних районах (в лесах и в оливковых рощах) на расстоянии до 10 км от меченых деревьев.

Было собрано 350 меченых мух обоих полов в 48 ловушках во время 15 проверок ловушек в период между 15 октября и 18 ноября. Меченые мухи, пойманные с 1 по 35 день после обработки деревьев индикатором, составляли 2% общего количества мух, отловленных в 48 ловушках или 0,15% общего количества мух, отловленных в 298 ловушках во всем районе Ровиес. 73% меченых мух попали в ловушки в течение первых 10 дней после обработки деревьев индикатором. Меченые мухи были обнаружены на расстоянии 44 300 м от места, где находились меченые деревья (максимальное расстояние). Радиоактивность меченых мух составляла от 258 до 9549 отсч/мин (фоновое излучение составляет 8 - 21 отсч/мин).

Эти предварительные эксперименты подтвердили существование более или менее постоянного местного перемещения (рассеивания) мух от полугорной рощи к близлежащим равнинам или к прибрежным оливковым рощам в районе Ровиес с севера на юг. Дальнее перемещение (миграция) мух Dacus в другие районы не было обнаружено. По-видимому, сосновые рощи являются барьером, препятствующим перемещению взрослых особей Dacus oleae.

ESTUDIO PRELIMINAR DE LA DISPERSIÓN DE MOSCAS DEL OLIVO (DACUS OLEAE GMEL.) MEDIANTE LA MARCACIÓN DE POBLACIONES NATURALES CON ^{32}P. Los autores de la memoria han realizado algunos ensayos preliminares para obtener datos sobre la dispersión de las moscas del olivo Dacus oleae Gmel. Estos ensayos se llevaron a cabo en los olivares de Rovies (Isla de Evía, Grecia) en otoño de 1961, época en que las moscas adultas abundan particularmente.

Para marcar la población natural se utilizó el ^{32}P de una mezcla de $H_3{}^{32}PO_4$ en HCl y proteína hidrolizada Staley No. 7. El 14 de octubre de 1961 se trataron con esta sustancia las ramas de 30 olivos de un olivar semimontañoso situado cerca de Rovies.

Las moscas se cazaron con 500 trampas McPhail en las que se emplearon como cebo fosfato diamónico al 3%. Las trampas se colocaron en los olivares de la región de Rovies y en zonas adyacentes (de bosque o de olivares) hasta una distancia de 10 km de los árboles tratados.

Durante los 15 recuentos efectuados entre el 15 de octubre y el 18 de noviembre se recogieron en 48 trampas un total de 350 moscas marcadas. Estas moscas, capturadas entre 1 y 35 días después del tratamiento, representaban el 2,8% del total de las moscas recogidas en las 48 trampas y el 0,15% del total de las moscas capturadas en 298 trampas colocadas en la zona de Rovies. El 73% de las moscas marcadas se capturaron durante los diez primeros días siguientes al tratamiento. Se encontraron hasta una distancia de 4300 m (dispersión máxima) de la zona donde se marcaron. Su radiactividad oscilaba entre 258 y 9549 impulsos/min y por mosca (actividad de fondo: 8 a 21 impulsos/min).

Estos ensayos preliminares han confirmado un movimiento local más o menos continuo (dispersión) desde el olivar semimontañoso hasta las llanuras adyacentes y los olivares de la costa (norte-sur). No se ha observado una migración de la mosca Dacus a otras regiones. Al parecer, los bosques de pinos actúan de barrera para los adultos de la Dacus oleae.

INTRODUCTION

It has long been known that many species of the family Tephritidae, which includes the olive fruit fly (Dacus oleae Gmel.), are capable of rapid wide-range flight. The Mexican fruit fly, Anastrepha ludens Loew, serves as an outstanding example in this connection. According to studies carried out in Texas in the United States of America, individuals of this species were trapped as far as 160 and 175 miles from the place of their breeding [3].

So far, the flight habits and migration of the olive fruit fly are poorly understood, although many research workers have dealt with this project for years. This lack of satisfactory results is attributed mainly to the inadequate experimental methods and techniques applied in the past. The somewhat arbitrary conclusions of most workers, so far from clearing up the whole matter, have rather led scientists to contradictory assumptions and theories.

The Italian entomologist LUPO, V. [5] carried out extensive investigations on the olive fruit fly migration and concluded that this species makes two seasonal migrations. The first migration, which he calls "summer migration", occurs during June and July, when flies move from mountainous regions (altitude 400 m and more above sea level) to the plains or to the coastal areas; the second or "autumn migration" occurs during September and October, when flies migrate from the coastal areas to the mountains. Both movements were related to the availability of fruits and the weather conditions.

Other research workers, such as BUA, G. [2], MELIS, A. [7], MARTIN, H. [6], SACANTANIS, K. [2] and ISAAKIDES, C.A. [4], recognize a seasonal migration of Dacus but disagree as far as the real causes of the movement are concerned. Some of these investigators attribute this movement to climatic conditions, principally temperature, precipitation or winds, while others ascribe it to the need of the insect to locate suitable fruits in which to lay eggs or its need of shelters for overwintering.

The lack of a method for artificially rearing the olive fruit fly for use in field tests has delayed high-level investigation of the subject in question. Because of this it was impractical for earlier researchers working in this field to use various materials and techniques for tagging the flies, such as marking with dyes.

The commercial production of radioactive isotopes, which have been recently introduced into applied biology, offers to the modern entomologist and biologist a valuable tool for investigation of the flight habits and the dispersal of fruit flies. Trials recently conducted in the United States with radioactive isotopes on species related to Dacus oleae Gmel., such as Dacus dorsalis Hendel, Ceratitis capitata Wied., and Rhagoletis completa Cresson, indicate that the use of isotopes with beta radiation, such as radioactive phosphorus (P^{32}), furnishes a standard technique for labelling a natural population of tephritid flies [1, 3, 11].

Besides having the advantages of accurate results and ease of application, these materials are not likely to cause adverse effects on the behaviour and habits of fruit flies.

An investigation of the flight habits and dispersal of the olive fruit fly by using radioactive P^{32} was the object of the preliminary trials reported

here. These were the first tests of this nature carried out in Greece with Dacus oleae.

These investigations, besides supplementing data already available concerning the biology and the habits of the olive fruit fly, were also considered to offer particular promise in pointing the way to more effective and economical control measures.

The subjects of investigation were (1) the flight-range of the olive fruit fly; and (2) the type and direction of movement. In respect of (2), a special effort was made to ascertain whether individuals of a natural Dacus population occurring in a certain olive-growing area exhibit dispersal (local movement of individuals within the limits of their habitat) or migration (a movement of the population outside the bounds of its habitat to another area and in a definite direction).

MATERIAL AND METHODS

Radioactive phosphorus (P^{32}) of $H_3P^{32}O_4$ in HCl was selected from among the internationally available radioisotopes and was used to label a natural population of Dacus existing in a semi-mountainous olive grove at Rovies, Evvia (Euboea). The use of the naturally occurring population was preferred over the field release of laboratory-reared and labelled individuals for the following reasons:

(1) The flight range of the native Dacus population which has already been adapted to the local environmental conditions may be more typical;
(2) The behaviour and the migration tendency of a reared or caged population may differ from that of the natural population; and
(3) It is difficult to obtain, under laboratory conditions, the large number of Dacus individuals required for studies of this kind.

The experimental area

A semi-mountainous olive grove (altitude 300 m above sea level) near the site known as "Chlio Nero" of Balanos' grove at Rovies, Evvia was selected for the application of the radioactive material used for labelling or tagging the natural fly population. This grove is surrounded on the north and north-east by the foothills of Mount Telethrion, which is largely covered by pine trees. This constitutes a natural barrier for the free movement of the olive fruit fly beyond. In all the other directions this grove is adjacent to Mr. Papadopoulos' olive grove, which extends on to the seashore (Fig. 1).

The selection of Balanos' field for carrying out these studies was decided for the following reasons:

(1) Data obtained from Dacus control experiments conducted in Papadopoulos' orchard during recent years [10] showed that a rather large and rapid increase of adult fly population occurred in this orchard by the end of September, even though the fruit infestation was low or very low. In such circumstances it might be supposed that new Dacus individuals came from Balanos' grove or other adjacent orchards that were by that time heavily infested.

(2) No chemical control had been applied on Balanos' grove during the previous three years. At the time these trials were carried out (October 1961) a heavy fruit infestation (85%) and a dense fly population (65-82 individuals per trap) occurred in Balanos' grove.

(3) The olive fruit of Balanos' orchard had prematurely ripened and was therefore unsuitable for ovipositions by females of the autumn brood, which thus would have to disperse in search of suitable fruit in which to lay their eggs. Furthermore, weather conditions, such as temperature, which drops substantially in the area under study in late autumn and in winter, and the northern and north-eastern winds prevailing there, might also favour the movement of flies from the semi-mountainous area (Balanos' orchard) to the coastal one (Papadopoulos' orchard).

Technique

A quantity of 40 mc of fresh radioactive P^{32} in the chemical form of PO_4 ion in HCl at a concentration of 5 mc per ml HCl, was imported from Canada*. The material (of radio-chemical purity 99.5% carrier-free) was shipped, as requested by us, in 4 bottles each containing 10 mc P^{32} (volume 2.00 ml). Shortly before its use the material was neutralized with normal $NaHCO_3$ until the solution became alkaline (pH 7.4). For this purpose approximately 5 ml of $NaHCO_3$ were used per ml of radioactive solution.

For labelling the natural adult fly population** occurring in the experimental field, the P^{32} solution was used in combination with Staley No. 7 protein hydrolysate. The flies feed upon this material and thus become radioactive. This protein has proved to have a satisfactory olfactory and gustatory attraction for Dacus flies [8, 9, 10]. This mixture of attractant and P^{32} solution was successfully used by BARNES, M. M. [1] to label a naturally occurring population of the walnut-husk fly, Rhagoletis completa Cresson. The senior author participated in the field trials carried out by Barnes in southern California during the fall of 1957.

The following formula was used in preparing the P^{32} bait solution:

1.	Staley No.7 hydrolysate protein	20.0 ml
2.	Radioactive P^{32} solution	12.5 ml
3.	Water	167.5 ml
		200.0 ml

A quantity of 600 ml of this preparation, containing a total of 30 mc radioactive P^{32}, was prepared.

Thirty olive trees were selected in the labelling station for the application of the P^{32} bait solution. On each tree, two twigs about 80 - 100 cm in length and equally developed were selected, one on the south-western and the other on the south-eastern side of the tree. They were treated on the morning of 14 October 1961. Care was taken in the application to insure that approximately 10 ml of solution was delivered per twig. The spray was applied with a small, laboratory air-pressure (pressure limit 200 lb)

* Atomic Energy of Canada Limited.

** Before these trials were carried out in the field, some tests were made in the laboratory with P^{32} bait solution at various concentrations to determine how Dacus adults responded to the beta radiation.

sprayer * . During the treatment favourable weather conditions prevailed in the field.

Precautions were taken during the handling of the radioactive material and the application of the spray, which was performed by the two first-named authors. Special attention was also paid to keeping persons and domestic animals away from the labelling operation.

For the collection of labelled flies and the determination of the Dacus flight range and movement, about 500 McPhail traps were hung on olive trees in the surrounding orchards and also on pine trees in adjacent areas. Traps were placed as far away as 10 km (in direct line) from the P^{32}-baited trees. A 3% solution of diammonium phosphate was used as a lure in the traps. The liquid was renewed at 5-d intervals. Approximately 380 traps were distributed in the nearby olive groves at Rovies (including semi-mountainous areas), while the remaining 120 traps were divided among more distant orchards of adjacent areas as far as Sipias, Limni. Some of these 120 traps were hung on pine trees of the forest area extending north-east of Rovies as far as the "Xiropigado" olive grove near Chronia (Fig. 1).

The traps were placed in zones at intervals of 200 - 300 m. The traps in each zone were specially marked so that they could easily be located and labelled flies trapped in them could easily be recorded.

Groups of experienced field workers were employed under the supervision of trained personnel to collect Dacus adults caught in the traps.

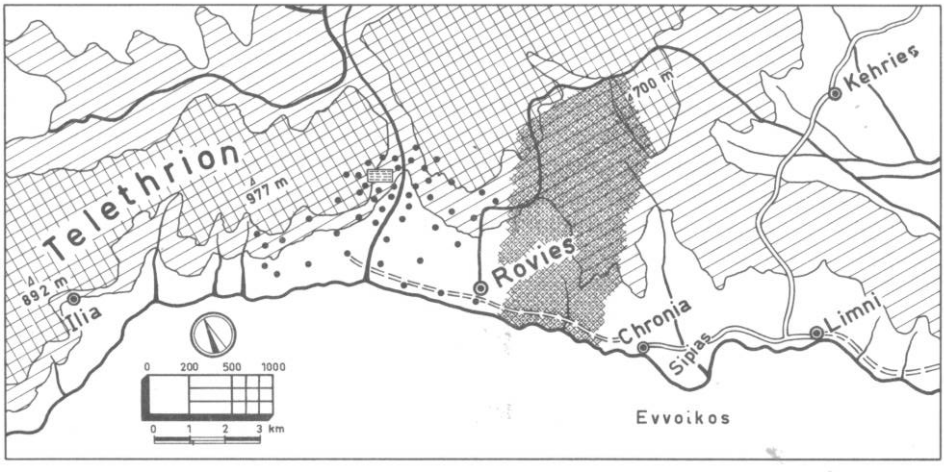

Fig. 1

Map of Rovies, Evvia (Euboea), showing P^{32}-baited trees and the positions of traps for labelled flies

P^{32}-baited trees

Traps for labelled flies

Pine-covered area

* "Sure shot", Milwaukee Sprayer Mfg. Co., Milwaukee.

Observations and counts of flies were made every other day. All insects collected in the traps were packed and forwarded to the Economic Entomology Laboratory of the Benaki Phytopathological Institute, Kiphissia, Athens.

RESULTS

To detect tagged flies and to determine their degree of radioactivity, a laboratory Geiger-Müller scaler or monitor (EKCO Electronics Ltd. No. 529) was used. For counts of less accuracy, such as those involving the determination of radioactivity of treated twigs, and for a quick on-the-spot observation of collected flies, a portable Geiger-Müller counter* was used.

Dacus individuals found in each trap were separately checked by the laboratory monitor after they had been carefully washed with distilled water on filter paper. To determine the true radioactivity of each labelled fly, the background radiation was taken into account.

The results obtained are shown in Table I. The data shown in this table together with additional observations permit the following conclusions.

The first labelled flies, 9 individuals collected in 7 traps, were detected 24 hours after treatment. Of these, 8 flies (3♀ and 5♂) were trapped 200 - 400 m away from the treated trees and 1 female fly about 2500 m away in a trap placed in the coastal section of Papadopoulos' orchard. The radioactivity of the first 8 flies ranged between 954 and 2099 counts/min per individual, while that of the ninth fly amounted to 273 counts/min (background radioactivity 8 - 12 counts/min).

A steady increase in the number of labelled flies occurred during the next 2 to 10 days following the treatment. Thereafter the figures declined substantially and the last tagged flies were collected on 18 November, that is 35 days after treatment.

Of a total of 7016 flies collected in 23 traps during the first ten-day period after treatment, 255 flies (3.6%) of both sexes (119♀ and 136♂) were labelled. Of these flies 88.2% were found in traps up to a distance of 1000 m away, 8.3% from 1000 to 2500 m (at Zervou, Kamini and Fytia) and the rest, 3.5%, from 2500 to 4300 m (at Kamini, Fytia and Osios David monastery). Three of the latter individuals were trapped on 24 October at a distance of 4300 m (maximum of flight range) in a trap placed in the Rovies olive grove. The radioactivity of 255 tagged flies ranged between 258 and 9549 counts/min per individual (background radioactivity 9 - 21 counts/min).

During the period under study a large increase in the adult fly population occurred in the whole Rovies area. Catches amounted to 76 914 flies in 293 traps (average 263 flies per trap).

In summary, during the period of observations (15 October - 2 December) 350 labelled flies were collected in 48 traps in which a total of 12 595 flies were trapped in the course of 15 counts made between 15 October and 18 November. Of the tagged flies representing a proportion of 2.8% of the above population, 86% moved up to a distance of 1000 m, 10% between 1000 and 2500 m and the remaining 4% moved up to a distance of 4300 m from

* Geiger-Müller counter TR 56 with window thickness of less than 4.0 mg/cm² (Panax Equipment Ltd.)

TABLE I

NUMBER OF LABELLED FLIES COLLECTED IN TRAPS PLACED AT DIFFERENT DISTANCES FROM THE LABELLING STATION

Date of collection	Time after treatment (d)	Number of labelled and normal flies collected in the same 48 traps									Number of flies collected in 75 traps		
		Up to 1000 m			1000 - 2500 m			2500 - 4300 m			4300 - 10000 m		
		Total collected	Labelled flies		Total collected	Labelled flies		Total collected	Labelled flies		Total collected	Labelled flies	
			(No.)	(%)		(No.)	(%)		(No.)	(%)		(No.)	(%)
15 October	1	450	8	1.8	-	-	-	169	1	0.6	2130	0	0.0
16 October	2	323	15	4.6	74	2	2.7	218	1	0.5	1535	0	0.0
18 October	4	645	38	6.0	103	6	5.8	-	-	-	4238	0	0.0
20 October	6	1951	73	3.7	517	4	0.8	172	1	0.6	2890	0	0.0
22 October	8	1955	80	4.1	266	4	1.5	320	4	1.3	5106	0	0.0
24 October	10	276	17	6.2	80	5	6.3	116	3*	2.6	3559	0	0.0
28 October	14	43	5	11.6	11	2	18.2	130	3	2.3	1201	0	0.0
30 October	16	19	2	10.5	-	-	-	-	-	-	1211	0	0.0
2 November	19	52	2	3.8	81	5	6.2	-	-	-	2600	0	0.0
4 November	21	905	22	2.4	-	-	-	-	-	-	2007	0	0.0
7 November	24	525	16	3.0	42	2	4.8	-	-	-	2344	0	0.0
10 November	27	71	2	2.8	241	3	1.2	19	1	5.3	1850	0	0.0
13 November	30	300	2	0.8	-	-	-	-	-	-	5005	0	0.0
16 November	33	2089	17	0.7	218	2	0.9	-	-	-	2584	0	0.0
18 November**	35	214	2	0.9	-	-	-	-	-	-	1291	0	0.0
		9818	301	3.0	1633	35	2.14	1144	14	1.2	39551	0	0.0

* Labelled flies trapped at the longest distance (4300 m) from the labelling station.

** Counts of catches continued up to 2 December 1961.

the labelling station. However, the above percentage (2.8%) for labelled flies becomes still lower (0.15%) if the estimate is based on the total flies collected in all traps used for the entire Rovies area. Total catches amounted to 234 373 flies caught in 298 traps during the same period.

The number of labelled flies might have been higher if two coverage sprays applied for the protection of the olive crop of Papadopoulos' orchard on 23 October and 4 November had not decreased the adult fly population.

It was only in the olive groves near Rovies that labelled flies were recovered. During these studies no tagged flies were found in any of the traps placed either in the forest area between Rovies and the olive orchards of Chronia, or between the latter and the olive groves at Sipias, Limni (Fig. 1).

In general, the results obtained from these preliminary studies showed that an unceasing movement or dispersal of Dacus individuals occurred from the semi-mountainous olive groves to the adjacent olive groves in the plain and to the coastal groves at Rovies during the period under study. Long-distance movement or migration of flies was not observed. The farthest distance at which labelled flies were recovered was approximately 4300 m (in direct line). Moving from the north, the flies followed mostly south-easterly and south-westerly directions. The technique used for labelling a naturally occurring Dacus adult fly population proved to be very effective.

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DISCUSSION

D.A. CROSSLEY: After the flies have fed on the P^{32} bait solution, how long do they retain detectable amounts of radioactivity? In other words, have you attempted to measure the biological half-life of P^{32} taken in through this bait solution?

C.E.D. PELEKASSIS: In our experiments labelled flies were recovered up to 35 d after treatment. Counts taken on the leaves of trees treated with P^{32} bait solution also indicate a biological half-life of about 30 d.

C.H. SCHMIDT: I was interested to note once again that apparently wind velocity had no effect on the migration of the labelled flies. You mentioned that the prevailing winds are north-east yet you found most of your flies in the south-eastern sector of the treated area.

C.E.D. PELEKASSIS: At the end of the period of observation, in the middle of November, there were some light winds which might have been favourable to movement in a south-easterly direction. But this could not be the sole factor involved. There must be some other explanation for the dispersal of the flies.

D.J. NADEL: I understand that the purpose of the experiments was to collect preliminary data on the movement and migration of Dacus from the test area, especially from the higher altitudes to the coastal regions.

There are several possibilities with regard to bait application methods:

(1) Single treatment with removal or isolation of the treated branches some set time after treatment - say, one day;

(2) Regular treatment twice a week, so as to ensure that an abundant supply of P^{32}-labelled bait is constantly available to the naturally occurring fly population;

(3) The method used in your experiments.

Why did you choose the third method, namely to treat only once and allow the bait to lose its attractiveness for the flies? What advantages do you feel were obtained? It would seem that a continuous series of treatments with the bait constantly available to the flies would have resulted in more complete data regarding fly movement, since there is then no question of bait deterioration.

C.E.D. PELEKASSIS: I do not know why Mr. Nadel has singled out just three methods of application. I think that various other possibilities could be conceived, depending mainly upon the ingenuity of the investigator and the purpose he has in mind.

There were three main reasons why we chose the technique we did. First, we had already had experience with a related technique. Secondly, owing to shortage of funds, import difficulties, etc., it was not possible to obtain more radioactive material at the time. Thirdly, we wanted to check the biological half-life of the radioactive material tested.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ И РАДИАЦИИ В БОРЬБЕ С НАСЕКОМЫМИ-ВРЕДИТЕЛЯМИ РАСТЕНИЙ И ЖИВОТНЫХ

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СССР

Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPES AND RADIATION IN ANIMAL AND PLANT INSECT PEST CONTROL. Crop-pest control is of major economic importance and demands the aid of the latest advances in science. Radioisotopes and radiation are being employed to increase the efficiency of existing insect pest control.

They are extremely valuable, since improvements to existing methods depend on having detailed data on the bioecology, toxicology, and so on. Radioactive labelling of insects has been extremely promising in bioecology; the labelling of grain pests (Eurygaster integriceps Put., Hadena sordida Skh.) and grain-pest parasites (Meniscus agnatus Crow, Pseudogonia cinerascens Rond.) has provided information about their areas of migration, habitats, sizes of population and the feeding habits.

The same technique was used to determine the rate of propagation of the Colorado beetle (Leptinotarsa decemlineota Say), which is subject to quarantine controls; subsequently an extermination programme was carried out on the basis of the data obtained.

It also provides a valuable means of studying the extremely complex problems of parasitism and predaceousness, in particular intermediate feeding cycles and chemotaxis. The feeding areas of field rodents have been mapped out with the help of a self-labelling, radioactive-bait technique.

Pesticides synthesized with radioisotopes have been used in conjunction with radiochromatography, fluorimetry and other techniques to study the highly complex biochemical processes caused to toxicants in plants and insects.

It has also been possible to determine the rate of hydrolysis of organic-phosphorus insecticide compounds of the thiphos and metaphos type as a function of the degree of development and the physiological state of plants as well as of environmental conditions. Data have been obtained on the length of time residual quantities of toxicants are retained in agriculture products following different periods of chemical treatment. Radioisotope techniques have yielded information on various metabolic processes exhibiting different degrees of stability when subjected to the action of herbicides.

Pesticides containing trace quantities of isotopes with short half-lives can be used as a means of judging the merits of various spraying systems for treating seeds and agricultural crops with pesticides; this method also provides information about the optimum doses.

Experiments have shown that ionizing radiations can be used to increase the virulence of insect nosophytes (Beauveria vassiana Unill.) and thus improve the efficiency of microbiological insect-pest-control methods. The sterilizing and lethal effects of ionizing radiations are also useful in combatting insect pests. Gamma-ray experiments have made it possible to establish sterilizing doses for a number of insect pests which attack crops and stored products (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophora malvella Hb., Leptinotarsa decemlineota Say, Chloridea obsoleta F.).

The data obtained in these investigations will be used to develop and improve the methods of insect-pest control used in agriculture.

EMPLOI DES RADIOISOTOPES ET DES RAYONNEMENTS DANS LA LUTTE CONTRE LES INSECTES NUISIBLES AUX PLANTES ET AUX ANIMAUX. Le problème de la lutte contre les parasites des cultures présente une grande importance pour l'économie nationale. Il importe, pour le résoudre, de tirer parti des réalisations scientifiques les plus récentes. Pour rationaliser les méthodes actuelles de lutte contre les insectes nuisibles et accroître l'efficacité de ces méthodes, on a fait appel aux radioisotopes et aux rayonnements.

Grâce à l'emploi des radioisotopes et des rayonnements, il est possible de rationaliser dans une large mesure les méthodes utilisées et d'augmenter leur efficacité. A cet effet, il est indispensable d'étudier à fond les problèmes d'écologie, de toxicologie, etc. Dans le domaine de l'écologie, la méthode du radiomarquage des insectes est particulièrement prometteuse. L'emploi de radioisotopes pour le marquage des insectes rava-

geurs des céréales (Eurygaster integriceps Put. et Hadena sordida Skh.) et de leurs parasites (Meniscus agnatus Crow et Pseudogonia cinerascens Rond.) a permis d'établir l'ampleur des migrations, de déterminer l'emplacement des foyers de reproduction et l'importance numérique des populations et d'étudier le mode d'alimentation des parasites.

Par la même méthode, on a établi la dynamique de la propagation du doryphore (Leptinotarsa decemlineota Say), ce qui a permis d'obtenir les données indispensables pour procéder à leur extermination.

En outre, cette méthode permet d'étudier diverses questions extrêmement compliquées ayant trait au parasitisme et au pillage (étude des cycles alimentaires de l'alimentation intérimaire et de la chimiotaxie). Grâce à l'emploi de la méthode d'automarquage des rongeurs des champs à l'aide d'appâts radioactifs, on a pu déterminer les dimensions des zones où ils se procurent leur nourriture.

L'utilisation de parasiticides marqués — conjointement avec les méthodes de radiochromatographie, de fluorométrie, etc. — a permis d'étudier les processus biochimiques les plus complexes qui interviennent dans les plantes et chez les insectes sous l'effet des substances toxiques.

Se fondant sur l'exemple des composés organiques du phosphore du type «thiophosphate» et «métaphosphate», on a déterminé la vitesse de l'hydrolyse de ces insecticides en fonction de la phase de développement de la plante, de son état physiologique et des conditions du milieu ambiant. On a également déterminé la durée de rémanence des résidus de substances toxiques sur les produits agricoles, en fonction de celle des traitements chimiques. Les radioisotopes ont aussi révélé les particularités des processus métaboliques qui s'effectuent chez les plantes qui résistent et celles qui ne résistent pas aux herbicides.

Le marquage des parasiticides à l'aide de radioisotopes de courte période permet d'évaluer l'efficacité du traitement des graines et semences dans différentes conditions de pulvérisation, et quels parasiticides conviennent le mieux pour un traitement donné.

L'action des rayonnements ionisants sur les micro-organismes entomopathogènes (Beauveria bassiana Unill.) a permis de démontrer la possibilité d'accroître leur virulence et, partant, d'améliorer la méthode microbiologique de lutte contre les insectes nuisibles. L'action stérilisante et létale des rayonnements ionisants peut être utilisée comme moyen actif de lutte contre ces insectes. On a déterminé les doses de stérilisation à l'aide des rayons gamma pour un certain nombre d'insectes ravageurs des récoltes stockées (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophota malvella Hb, Leptinotarsa decemlineota Say, Chloridea obsoleta F.) et des semences.

Les données obtenues à la suite des recherches permettront de mettre au point et de perfectionner les méthodes de lutte contre les insectes nuisibles, notamment à l'agriculture.

ИСПОЛЬЗОВАНИЕ РАДИОИЗОТОПОВ И РАДИАЦИИ В БОРЬБЕ С НАСЕКОМЫМИ-ВРЕДИТЕЛЯМИ РАСТЕНИЙ И ЖИВОТНЫХ. Проблема борьбы с вредителями сельскохозяйственных культур имеет крупное народнохозяйственное значение. При разрешении этой проблемы особое значение приобретает использование последних достижений науки. В целях рационализации и повышения эффективности существующих методов борьбы с вредными насекомыми были использованы радиоактивные изотопы и излучения.

Использование радиоактивных изотопов и излучений является могучим средством рационализации и повышения эффективности существующих методов борьбы с вредными насекомыми. Разработка этих методов вызывает необходимость детального изучения вопросов биоэкологии, токсикологии и т.д. В области биоэкологии весьма перспективным является метод радиомаркировки насекомых. Применение радиоизотопов для маркировки насекомых вредителей зерновых культур (Eurygaster integriceps Put, Hadena sordida Skh.) и их паразитов (Meniscus agnatus Crow, Pseudogonia cinerascens Rond.) позволило установить размеры миграций их, определить места резерваций, численность популяций и изучить вопросы питания указанных паразитов вредных насекомых.

Этим же методом была установлена динамика распространения карантинного объекта картофельного жука (Leptinotarsa decemlineota Say), что позволило получить необходимые данные для проведения истребительных мероприятий.

Кроме того, метод дает возможность исследовать сложнейший комплекс вопросов паразитизма и хищничества (вопросы изучения пищевых циклов промежуточного питания и хематаксиса). Применение метода самомаркировки полевых грызунов с помощью радиоактивных приманок позволило установить размеры пищевых ареалов.

Использование пестицидов, синтезированных с радиоактивными изотопами, в сочетании с методом радиохроматографии, флюрометрии и другими позволило исследовать сложнейшие биохимические процессы, протекающие в растениях и насекомых под влиянием токсикантов.

На примере фосфорноорганических соединений типа тиофоса и метафоса была установлена скорость гидролиза этих инсектицидов в зависимости от фазы развития растения и его физиологического состояния, а также условий внешней среды. Определена длительность сохранения остаточных количеств токсикантов на сельскохозяйственной продукции в зависимости от сроков химических обработок. С помощью радиоактивных изотопов выявлены особенности метаболитических процессов, устойчивых и неустойчивых к гербицидам растений.

Внесение в пестициды изотопов с коротким периодом полураспада в индикаторных количествах дает возможность производить оценку качества обработки семенного материала и посевов сельскохозяйственных растений пестицидами при использовании различных систем опрыскивателей, а также определять оптимальные нормы их расхода.

Путем воздействия ионизирующих излучений на энтомопатогенные микроорганизмы (Beauveria bassiana Unill.) доказана возможность повысить их вирулентность в целях улучшения микробиологического метода борьбы с вредными насекомыми. Стерилизующее и летальное действие ионизирующих излучений может быть использовано как активное средство борьбы с вредными насекомыми. Применением гамма радиации установлены стерилизующие дозы для ряда насекомых-вредителей запасов (Calandra granaria L., Acanthoscelides obtectus Say, Pectinophora malvella Nb., Leptinotarsa decemlineota Say, Chlorides obsoleta F.), а также посевов.

Полученные данные исследований будут положены в основу разработки и усовершенствования методов борьбы с вредными насекомыми в условиях сельскохозяйственного производства.

UTILIZACIÓN DE LOS RADIOISÓTOPOS Y DE LAS RADIACIONES EN LA LUCHA CONTRA LOS INSECTOS NOCIVOS PARA LAS PLANTAS Y LOS ANIMALES. El problema de la lucha contra los insectos dañinos es de gran importancia para las economías nacionales. Los últimos descubrimientos de la ciencia pueden contribuir considerablemente a resolverlo. A fin de emplear de una manera más racional y eficaz los métodos de lucha contra las plagas de insectos, se ha recurrido a los radioisótopos y a las radiaciones.

Los radioisótopos y las radiaciones son auxiliares muy útiles para mejorar los métodos de lucha contra los insectos dañinos. Su empleo requiere un estudio detallado de toda una serie de cuestiones de bioecología, toxicología, etc. En bioecología, la marcación de los insectos es una técnica muy prometedora. El empleo de los radioisótopos a fin de marcar a los insectos dañinos para los cultivos de cereales (Eurygaster integriceps de los radioisótopos a fin de marcar a los insectos dañinos para los cultivos de cereales (Eurygaster integriceps Put, Hadena sordida Skh), y a sus parásitos (Meniscus agnatus Crow, Pseudogonia cinerascens Rond) ha permitido calcular la extensión de las migraciones, delimitar los reservorios, evaluar la importancia de las poblaciones y estudiar el modo de alimentación de los parásitos de los insectos nocivos.

Con este método se ha podido establecer la dinámica de la dispersión del escarabajo de la patata (Leptinotarsa decemlineota Say) y obtener los datos indispensables para aplicar medidas para su erradicación.

Se han podido también investigar los complicadísimos problemas del parasitismo y la predación (estudio de las cadenas alimentarias de la alimentación intermedia y de la quimiotaxia). Gracias a la automarcación de los roedores mediante cebos radiactivos se puede determinar la importancia de las zonas donde éstos se alimentan.

La utilización de los pesticidas marcados, en combinación con la radiocromatografía, la fluorometría y otras técnicas, ha permitido investigar los procesos bioquímicos más complejos que tienen lugar en las plantas y en los insectos bajo la acción de agentes tóxicos.

Se ha calculado la velocidad de hidrólisis para los compuestos fosforoorgánicos del tipo Tiofos y Metafos en función de la fase de crecimiento de las plantas, de su estado fisiológico y de las condiciones del medio ambiente. Se ha determinado el tiempo que los residuos de agentes tóxicos permanecen en los productos agrícolas en función de la época en que se procedió al tratamiento químico. Con ayuda de los radioisótopos se han investigado las características de los procesos metabólicos de las plantas resistentes y no resistentes a los herbicidas.

La marcación de los pesticidas con isótopos de período corto ha permitido estudiar cualitativamente el tratamiento de las semillas y de las plantas utilizando diferentes sistemas de aplicación de pesticidas pulverizados y establecer normas óptimas para su empleo.

Mediante la acción de las radiaciones ionizantes sobre los microorganismos entomopatogénicos (Beauveria bassiana Unill) se ha demostrado la posibilidad de aumentar su virulencia y, por tanto, mejorar los métodos microbiológicos de lucha contra las plagas de insectos. La acción esterilizadora y letal de las radiaciones ionizantes puede ser muy eficaz en la lucha contra las plagas de insectos. Utilizando rayos gamma se han establecido las dosis esterilizadoras para toda una serie de insectos nocivos para los productos agrícolas almacenados

(Calandra granaria L., Acanthoscelides Obtectus Say, Pectinophora Malvella Hb., Leptinotarsa decemlineata Say, Chloridea obsoleta F.) y los cultivos.

Los datos obtenidos con esas investigaciones servirán de base para elaborar y perfeccionar los métodos de lucha contra los insectos dañinos para la agricultura.

В Советском Союзе проводится интенсивная исследовательская работа по использованию радиоактивных изотопов и излучений в целях разработки теоретических и практических задач борьбы с вредными насекомыми. В этих исследованиях значительное место отводится изучению вопросов токсикологии, биоэкологии вредных насекомых и их паразитов, а также исследованию процессов, связанных с рационализацией методов борьбы.

Применение радиоактивных изотопов позволило по новому подойти к решению поставленных задач и выявить данные, которые не могли быть получены обычными методами исследования.

Наряду с этим приводятся также исследования по применению мощных источников ионизирующих излучений для целей активной борьбы с вредными насекомыми.

Правильная организация мероприятий по борьбе с вредителями возможна лишь на основе детального изучения особенностей их биологии (миграций, ареалов распространения, мест резерваций и ряд других вопросов).

Для изучения указанных вопросов успешно используется метод маркировки насекомых радиоактивными изотопами, который позволяет вести наблюдения над большим числом объектов. Это дает возможность получать более достоверные результаты, используя метод математического анализа.

Метод радиомаркировки нашел применение в паразитологии человека [1 - 6] и животных. В этом плане изучение характера и предельной дальности перемещений насекомых имеет большое значение для понимания их роли как переносчиков возбудителей инфекционных заболеваний человека и животных.

Одним из наиболее опасных вредителей сельскохозяйственных животных являются вольфартовые мухи (Wohlfahrtia magnifica Schin.), личинки которых паразитируют в ранах домашних животных. Зона распространения этих мух - южные районы Советского Союза.

Миазы, вызываемые личинками, приносят большой вред овцеводству. Эффективная борьба с вольфатиозом скота без знания биоэкологии мух весьма затруднительна. В связи с этим для изучения некоторых биологических особенностей биологии этих мух был применен метод радиомаркировки. Маркировка мух производилась путем скармливания им сахарного сиропа, содержащего радиоактивный изотоп фосфора с концентрацией 1,86 микрокюри в 1 мл. Маркированные мухи в количестве нескольких тысяч экземпляров были выпущены в местах выпаса овец. В дальнейшем производился отлов мух ловушками с мясными приманками, затравленными 0,02% раствором хлорофоса, с последующим анализом отловленных мух радиометрической аппаратурой. Ловушки расставлялись в зоне выпаса скота на расстоянии от 150 м до 10 км от места выпуска мух. Наибольшее количество мух было отловлено у водопоя, который находился на рас-

стоянии 2 км от места выпуска. Это позволило сделать вывод, что общий водопой является местом резервации мух и перелета их с одних отар на другие. Лабораторный и полевой опыты также показали, что у маркированных мух наблюдается трансларвальный переход радиоактивного фосфора от самок к личинкам. Последнее позволяет установить размеры распространения инвазии от отдельных особей [7]. Метод радиомаркировки может быть также применен при изучении миграций мелких объектов: тлей (Aphididae), трипсов (Thysanoptera), клещей (Acarina), блох (Aphaniptera) и др. В этом случае, помимо радиометрического анализа, может быть использован метод радиоавтографии. Радиоавтограммы насекомых, полученные при длительном (24 - 72 часа) экспонировании, дают отчетливую картину наличия радиоактивной метки в них. Указанный метод радиоавтографии был применен в исследованиях миграции блох (Xenopsilla heopis и Ceratophyllus fosciatus), паразитирующих на грызунах и являющихся переносчиками ряда опасных заболеваний.

Для осуществления маркировки блох производилась маркировка радиоактивным изотопом мышей, на которых эти блохи паразитируют. При маркировки мышам путем инъекции вводился препарат радиоактивного фосфора из расчета 4 микрокюри на 1 г веса животных. После выпуска маркированных мышей, в места их обитания на различных расстояниях от места выпуска производился массовый отлов мышей. После обработки мышей и находящихся на них насекомых, летальной дозой наркоза, собранные насекомые проверялись радиоавтографическим методом. Проведенные опыты позволили установить размеры зоны распространения блох от мышей, инъекцированных радиоактивным фосфором [8].

В исследованиях по защите растений, маркировка насекомых радиоактивными изотопами, была применена при изучении миграций опасного вредителя зерновых - клопа черепашки (Eurigaster integriceps put.), зерновой совки (Hadena sordide pxh), а также паразитов последней (pseudogonia cinerascens и Meniscus agnatus grov) [9 - 11]. Маркировка этих насекомых производилась различными специально разработанными методами: в личиночной стадии насекомые метились путем кормления их на пище, содержащей радиоактивные изотопы. Взрослые насекомые с твердым хитиновым покровом, метились путем погружения их в радиоактивные растворы.

Для маркировки бабочек и паразитических мух, во избежании возможных нарушений их покровов, применялся метод самомаркировки, заключающийся в использовании положительного эффекта фото- и хемо таксисов. Насекомые, привлеченные светом или запахом к приманкам, содержащим радиоактивные вещества, соприкасаясь или питаясь ими, становились маркированными.

Изучение особенностей биологии вредителей картофеля - колорадского жука (Leptinotarsa decemlimata Say) методом маркировки позволило изучить миграции перезимовавших жуков.

Выяснено, что после зимовки жуки, только что вышедшие из почвы, способны наряду с передвижением по земле совершать сравнительно большие перелеты, дальность которых достигает 500 и бо-

лее метров в сутки. Наибольшую активность жуки проявляют в период спаривания, пролетая за сутки более 1 км. В этот период происходит максимальное расширение площадей заражения. Во время яйцекладки подвижность жуков резко снижается, и маркированные жуки перемещаются 10 - 40 м в сутки.

С помощью маркировки радиоактивными изотопами была проверена существующая методика обнаружения очагов заражения и плотности их заселения жуками. В результате проведенных контрольных обследований было установлено, что даже при трехкратном обследовании, проводимых одно за другим посредством ручных сборов, обычно обнаруживается не более 80% жуков, находящихся на данном поле. Это позволило обосновать, в известной мере, причины частичного обнаружения очагов колорадского жука и объяснить причины образования многолетних очагов.

Применение радиоактивных изотопов, являющихся источником жесткого гамма-излучения (Co^{60},Fe^{59}), дает возможность проводить наблюдения также и за миграциями скрытноживущих насекомых, обнаруживая с помощью радиометрических приборов под слоем коры короедов различных видов, а в почве - проволочников и др. [12]. Маркировка радиоактивными изотопами растений в период их цветения позволила решить вопрос о преимущественном и избирательном питании вредных насекомых и ряда их паразитов различными видами растений [13]. Кроме того, применение радиоактивных изотопов позволяет определять пищевые циклы, устанавливать хищничество и паразитизм (т.е. выбор хищниками и паразитами жертвы и хозяина).

Наряду с исследованиями в области прикладной энтомологии представляет практический интерес применение метода самомаркировки теплокровных биоценоза. Опыты проводились по оценке приманочного метода борьбы против грызунов, а также влияния этого метода на полезную фауну (промысловых птиц и зверей).

Метод исследования заключался в следующем. В местах предполагаемого обитания вредных грызунов разбрасывались отравленные приманки. В качестве приманок использовался овес с добавлением в него вторацетата бария и радиоактивного раствора $Na_2HP^{32}O_4$ из расчета 300 г приманки на 1 га.

Посещения грызунами мест обитания подтверждалось обнаружением экскрементов и трупов особей, питавшихся отравленными приманками, содержащими радиоактивный фосфор. Дозиметрия трупа являлась единственным средством установить, что животное погибло от отравленной приманки, так как обнаружить яд в трупном материале химическим путем не в полевых условиях было невозможно.

Проверка опасности отравленных приманок для промысловых птиц показала, что только незначительный процент отстреляных птиц содержал следы радиоактивности. Наряду с этим метод самомаркировки позволяет установить предпочитаемость грызунов в выборе типа кормовой основы приманки, в частности было установлено, что для суслика наилучшей приманкой является овес, а для водяной крысы - кукуруза.

Применение метода самомаркировки грызунов и промысловых животных радиоактивными изотопами дает возможность изучать слож-

ный комплекс вопросов теплокровных биоценоза и установления кормовых ареалов [14].

При проведении химической обработки сельскохозяйственных растений существенное значение имеет: а) определение качества обработки полей, б) установление оптимальных норм расхода пестицидов, в) определение сравнительных характеристик опрыскивателей различных систем.

Для решения этих вопросов был разработан новый метод, основанный на использовании радиоактивных изотопов.

О результатах применения метода более подробно было доложено в 1960 г. на симпозиуме в Бомбее, посвященным вопросам применения радиоактивных изотопов и радиации в энтомологии [15, 16]. В настоящее время этот метод используется в научно-исследовательской практике при проверке различных систем опрыскивателей при наземном и авиационном методах обработке посевов полевых культур, виноградников и садов.

Исследование сложнейших вопросов токсикологии насекомых и интоксикации растений (метаболических процессов, связанных с поступлением токсикантов в организмы, определение токсических характеристик пестицидов, длительности их сохранения, установление остаточных количеств токсических соединений в сельскохозяйственной продукции и многие другие) невозможно без привлечения общепризнанного в мировой практике метода радиоактивных индикаторов. Методом радиоактивных индикаторов получены данные о различной скорости проникновения пестицидов в организмы растений, вредных насекомых и грызунов; прослежена динамика распространения, пестицидов кишечного и контактного действия внутри организма насекомого, а также преимущественная локализация пестицидов в отдельных органах и тканях.

Исследования с помощью радиоактивных изотопов позволили выяснить ряд вопросов, связанных с практикой применения фосфорорганических соединений типа тиофоса и метафоса, а также изучить поведение этих химикатов в растениях, установить скорость гидролиза препаратов у различных растений в зависимости от условий их выращивания, а также определить остаточные количества токсикантов в сельскохозяйственной продукции. Методом радиохромотографии установлено, что тиофос и его метильный аналог обладают весьма слабым системным действием [9].

В практике применения инсектицидов в борьбе с вредными насекомыми недостаточно уделяется внимания влиянию пестицидов на растения при различных условиях их питания. Между тем, изменением условий питания, как подтверждают проведенные исследования, представляется возможным снизить степень токсического действия яда на защищаемое растение, особенно при неблагоприятных условиях произрастания. Применением метода радиоактиных индикаторов удалось установить, что хлорорганические препараты (ГХЦГ и ДДТ) оказывают существенное влияние на поступление фосфора в растение.

Так, например, при стимулирующих дозах хлорорганических препаратов процесс поступления P^{32} в растительные ткани активировался, и радиоактивный фосфор концентрировался в формирующихся органах в 1,5 - 2 раза интенсивнее, чем при поступлении его в необработанные токсикантом растения. Подобная активация поглощения радиоактивного фосфора является следствием усиления обмена веществ, вызываемого влиянием препарата. При воздействии же ингибирующих доз инсектицида наблюдалось нарушение нормального поступления фосфора P^{32} в растение, что явилось результатом угнетения токсикантом процессов жизнедеятельности.

Увеличение продуктивности отдельных органов и растения в целом было получено при совместном применении хлорорганических препаратов с фосфатами.

Совместное применение хлорорганических препаратов с фосфатами проверено в производственных условиях на различных культурах: пшенице, картофеле и других.

Таким образом, рациональное применение физиологически активных химических средств в сочетании с определенными удобрениями в практике защиты растений может обеспечить не только защиту культуры от вредных насекомых, но и дополнительно повысить урожай от 10 до 20 и более процентов [18].

В последнее время, как в СССР, так и в других странах большое внимание уделяется разработки методов лучевой дезинсекции продуктов запаса, а также лучевой стерилизации полевых вредителей в целях снижения численности их популяций.

Вопросу дезинсекции продуктов при их хранении посвящена довольно обширная литература. Рядом авторов [19, - 25] были проведены исследования по изучению действия ионизирующих излучений на насекомых вредителей запасов (Calandra granaria L., Calandra oryzae L., Acanthoscelides obtectus Say, Bruchus prisorum L).

Исследования летального действия ионизирующих излучений показали, что хотя гибель насекомых непосредственно под лучем наступает при 300 тыс. р, однако и значительно более низкие дозы (30 тыс. р) приводят к резкому сокращению продолжительности жизни насекомых.

При проектировании и осуществлении гамма-дезинсекторов большой производительности особое значение приобретает выбор оптимальной мощности источника облучения, соответствующей экономически наиболее целесообразному варианту. В связи с этим возникает необходимость в выборе метода облучения, обеспечивающего достаточную дезинсекцию при минимальных затратах на сооружение дезинсекторов и их эксплоатацию. Значительный выигрыш в этом отношении может быть получен за счет применения метода половой стерилизации насекомых. Дозы, необходимые для половой стерилизации насекомых, значительно ниже летальных.

Многочисленными исследованиями, проводимыми как в СССР, так и в других странах с различными вредителями запасов зерна, установлено, что оптимальными дозами для стерилизации насекомых вредителей являются дозы в 7 - 10 тыс. р. Эти данные явились исход-

ными при проектировании гамма-дезинсекторов. В настоящее время уже разработан ряд проектов гамма-дезинсекторов, часть которых осуществляется [26, 27].

В указанных дезинсекторах использованы различные приемы стерилизации. Одним из подобных решений является подача зерна к облучателю в контейнерах. В качестве источников гамма-излучения используются радиоактивные изотопы Co^{60} и Cs^{137}.

Наиболее рациональным является использование принципа облучения зерна при его движении непрерывным потоком мимо стержневого излучателя. Этот способ дезинсекции был положен в основу расчета гамма-дезинсектора зерна производительностью 30 т/час при суммарной активности источника излучения (Co^{60}) 60 кг эквивалент радия. На рис.1 показана схема подобной установки. Отличительной особенностью этого гамма-дезинсектора является применение водяной защиты и заглубление всего дезинсектора в грунт.

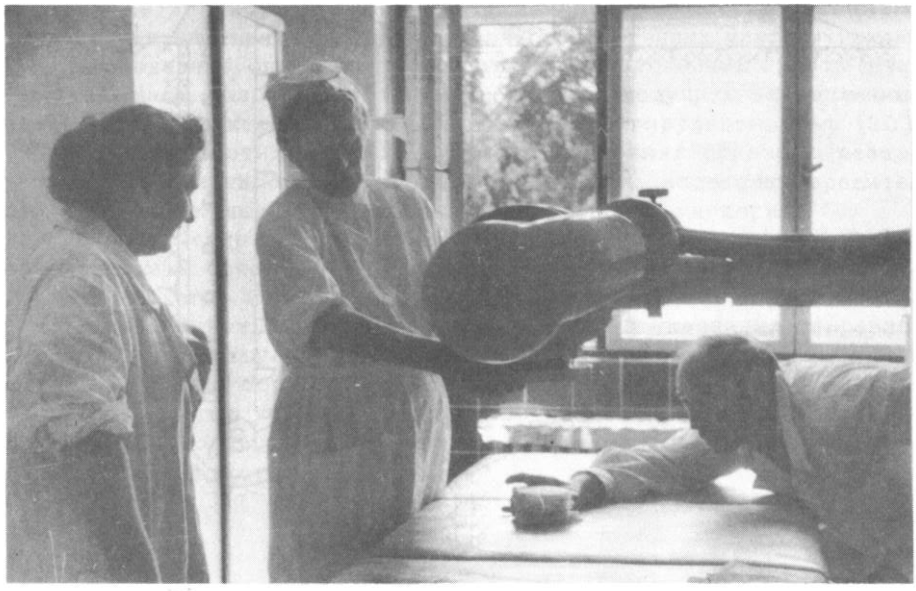

Рис.1

Подготовка к опыту по облучению куколок колорадского жука на рентгеновской установке, мощностью 200 - 650 р/мин.

В области борьбы с вредителями полевых культур в последние годы особый интерес энтомологов многих стран вызывает использование методов химической и лучевой стерилизации, как средства снижения численности естественной популяции.

Метод лучевой стерилизации позволяет значительно сократить или полностью исключить применение химических средств борьбы и, следовательно, устранить вредное действие на человека и сельско-

хозяйственных животных инсектицидов, остающихся в сельскохозяйственных продуктах после их обработки. Кроме того, этот метод является единственным, когда инстинктивное стремление насекомых к увеличению популяций направляется и используется человеком на самоуничтожение вредителя как вида.

В связи с этим в Советском Союзе проводятся поисковые исследования по оценке указанного метода на ряде объектов: хлопковой совке (Chloridae obsoleta F.), капустной мухе (Chortophila

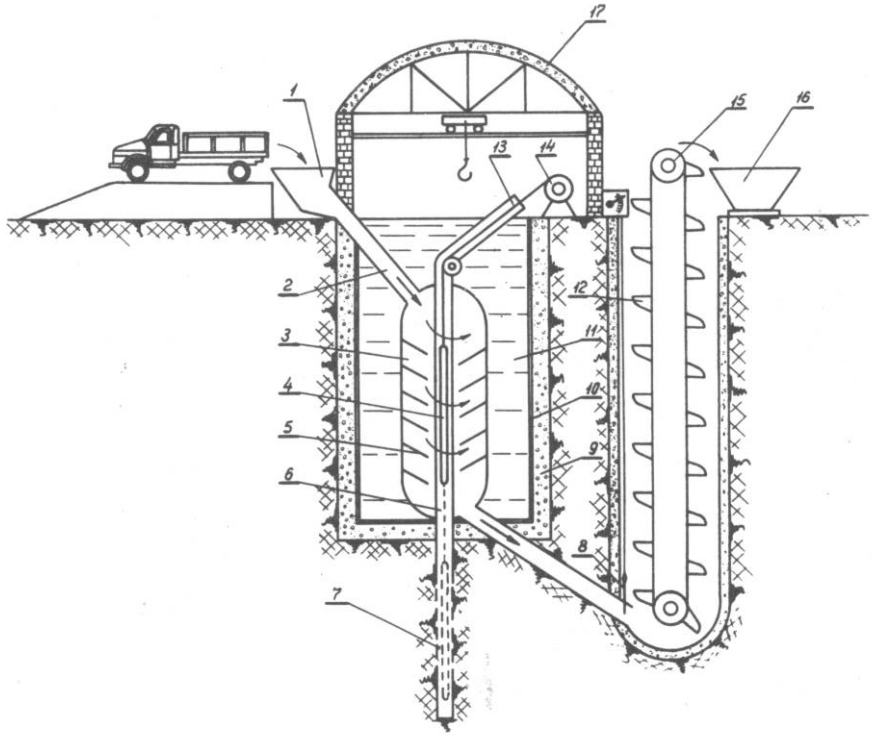

Рис.2

Принципиальная схема гаммадезинсектора зерна:
1 - приемный бункер; 2 - зернопровод; 3 - камера облучения; 4 - стержневой гамма-излучатель; 5 - спиралеобразные поверхности; 6 - металлическая труба; 7 - скважина; 8 - шибер; 9 - бетонированный колодец; 10 - слой гидроизоляции; 11 - вода; 12 - ковш транспортера; 13 - трос; 14 - лебедка; 15- разгрузочный транспортер; 16 - приемный бункер.

brassicae Bouche), колорадском жуке (Leptinotarsa decemlimata), мальвовой моли (Pectinaphora malvella Hb.).

Опыты по стерилизации этих насекомых показали, что стерилизующие дозы лежат в пределах 6 - 10 тыс. рентген [28, 29]. На рис.2 показана подготовка к облучению куколок колорадского жука на рентгеновском аппарате.

Проводятся исследования по изучению поведения стерилизованных особей в природе и определению их активности по сравнению с необлученными насекомыми, что необходимо для установления оптимальных соотношений между стерилизованными и нестерилизованными самцами в природе.

Особенное значение приобретает вопрос об установлении сравнительной эффективности метода при наличии у насекомых моно- или полигамии, а также при ограниченном числе генераций в году. Ионизирующие излучения являются также эффективным средством воздействия на микроорганизмы. Излучения могут быть успешно использованы в практике для борьбы с возбудителями заболеваний растений, а также для повышения вирулентности энтомопатогенных микроорганизмов.

Проведенные опыты по облучению энтомопатогенных грибов (Beaveria bassiana, Aspergillus flavus) позволили получить закрепленные изменения, характеризирующиеся повышенной вирулентностью [30].

Испытание этих штаммов в полевых условиях показало резкое увеличение смертности зараженных или вредных насекомых вредителей зерновых культур в местах их массового размножения.

В заключение следует отметить, что в моем кратком сообщении освещены лишь основные направления исследований в области использования изотопов и радиации в целях борьбы с вредными организмами.

О повышенном интересе к исследованиям в указанных направлениях свидетельствует организация 2-го симпозиума, посвященного острой проблеме борьбы с вредными насекомыми.

Содружество ученых многих стран является залогом успешного разрешения проблемы практического использования атомной энергии в сельском хозяйстве.

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DISCUSSION

K. K. NAIR (Chairman): In your paper you have referred to the grain irradiator recently fabricated in the Soviet Union. Could you tell me what exactly is the cost of operation of this unit? How much, that is, does it cost to irradiate one ton of grain at the proposed throughput of 30 t/h?

S. V. ANDREEV: The economic calculations have not yet been made, but it will be considerably cheaper if the irradiator is used in stationary conditions, close to a large silo where grain is kept for a long time. It is difficult to give the exact cost per ton. A number of plants are at present being set up in our country for the sterilization of grain and food products.

On the basis of the studies to be carried out once these plants are completed, an evaluation of their effectiveness and economic viability will be made. The plant I mentioned as one of the main ones should prove economically profitable.

M. S. QURAISHI: May I request you to throw some more light on the study of Eurygaster integriceps carried out in the Soviet Union?

S. V. ANDREEV: Study of the migration of Eurygaster integriceps has been carried out in the southern part of the Soviet Union. First larval migration, both vertical and horizontal, was studied under different climatic conditions. The larvae were reared on plants growing on soil sprayed with a solution of $Na_2H^{32}PO_4$. By labelling the larvae we were able to establish migration to field crops and wheat. We also found weather-dependent vertical migration; when conditions were favourable the larvae rose to the tops of plants, and when temperatures increased above normal they went into the ground and remained there until the next favourable period.

We also studied the migration of the adult insects. In summer they were collected in large numbers from grain by special mechanical collectors and then, by means of a special long stick with a hole in it, they were dipped in a tank containing a $Co^{60}Cl_2$ aqueous solution. After 1 min they were extracted and placed to dry in another tank filled with straw. This second tank was carried into the field and the insects were released within a given area.

Their radius of flight was then checked with field counters. It was found that in their final stages of development the insects collected in wooded areas in autumn for hibernation.

In spring, after over-wintering, they flew out of the forests, and during the early period, when their fat reserves were exhausted, were to be found at first close to the young wheat. During this period they stayed on the wheat for a few days and then when they had recovered their strength to some extent, began to carry out longer flights. We found that such flights may range over many tens of kilometres when there is no wind.

All their flight ranges were determined both in spring and autumn. We established the strength and direction of migratory flights at the various ecological stages and formulated techniques for control in these regions.

M. S. QURAISHI: I was unable to follow just when E. integriceps was labelled If I heard correctly they were labelled after hibernation and then released in the field.

If they were labelled at the time of migration from mountains to field, may I know the advantages of using a long-lived isotope like Co^{60}?

S. V. ANDREEV: The insects were labelled at the beginning of spring, on emerging from the forests. Afterwards, during the summer, we observed the generation which had been hibernating. Then the larvae appeared, non-labelled of course. After a certain period we obtained the adult insects from these and collected a new generation, which we again labelled in the spring or early summer. The reason we used Co^{60} was to be able to detect those hidden under leaves or topsoil in the forest, also to extend the observation period, in order to observe those emerging in the spring. The insects move about a good deal under the leaves, so that much of their radioactivity might have been lost if other techniques had been applied.

H. J. de FLUITER: In his introduction, the speaker mentioned Soviet investigations on the migration of Aphididae and Thysanoptera. We are very much interested in the investigations into the migration of Thysanoptera. Did these investigations deal with the migration of the adult thrips or with the migration of the larvae into the soil, where they often hibernate at various depths? How did you label the thrips and which species are involved?

S. V. ANDREEV: In addition to Eurygaster integriceps we have studied the migration of Hadena sordida and Pseudogonia. Our investigations have in fact been mainly concerned with soil pests. The soil pests are marked with metallic cobalt introduced into the anus. Vertical and horizontal migration is studied with a special counter and the vertical migration is studied according to absorption in the soil, so that the thickness of the layer under which the insect is to be found can be read. Horizontal migration is studied from the insect's range of movement over the surface of the soil, also by using a counter.

For the purpose of studying the migration of thrips on the cotton plant, they were labelled by being fed on P^{32} through the plant. Later, thrips collected from other plants in the neighbourhood of the experimental ones, and also from different plants from those used in the experiment, were transferred on to film on which they were exposed for 24 - 72 h. This made it possible to determine the extent of migration over 24 h, and also throughout their lifetime.

B. DARIS: On the subject of irradiation of grain in silos, I would like to ask how many curies does the Co^{60} source which you used give?

S. V. ANDREEV: In the main gamma-disinfestation source I have described, the entire estimate was based on an overall activity of 60 kg Ra equiv.

B. DARIS: How long does it take the grain to move from the entrance to the exit?

S. V. ANDREEV: At a throughput of 30 t/h the grain was no longer in the irradiation area than one minute.

R. KLJAJIĆ: You say you are studying questions of plant physiology as affected by the use of pesticides. This problem has been a frequent subject in modern phytopharmaceutical studies and modern scientific development has made possible the application of precise methods and criteria for the determination of plant behaviour in relation to pesticides. Classical methods, useful enough though they may be from the point of view of agricultural practice, do not always provide an objective and comprehensive picture of the most subtle phenomena in plants.

Research in our laboratory is aimed at applying more precise methods to the study of the effect of certain pesticides upon the living processes of treated plants.

In our paper: "Contribution to the study of P^{32} absorption in soybean and maize plants depending on application of some pesticides" *, by Kljajić R., Štrbac N., Šinžar B. and Ostojić N., certain data are given concerning soya beans and maize.

Soya bean varieties (Dickmans Grüngelbe, and Manchor Wisconsin) have been grown in sand culture in laboratory conditions (T = 20-22°C and

* Submitted to but not presented at the Symposium.

5000 lux). Treatment by pesticides, Kelthane W (conc. 0.0250 a.i.%; 5000 lux). Treatment by pesticides, Kelthane W (conc. 0.0250 a.i.%; 0.0500 a.i.%; 0.0650 a.i.%, Metasystox (0.0150 a.i.%; 0.0250 a.i.%; 0.0625 a.i.%) and Zineb WP (conc. 0.0975 a.i.%; 0.1300 a.i.%; 0.1625 a.i.%) has been carried out.
0.0625 a.i.%) and Zineb WP (conc. 0.0975 a.i.%; 0.1300 a.i.%; 0.1625 a.i.%) has been carried out.

Maize has been treated with Hg (MEMS 1.5% a.i.) and Hg (MEMS 1.5% a.i.) + Lindane (20% a.i.) pesticides.

All the results obtained for the absorption of P^{32} have been subjected to statistical analysis. They show that there are considerable differences in the effects of pesticides used upon the living processes of treated soya beans and maize. The degree of effect depends on the pesticide applied, its concentration, the kind or variety of plants investigated and the phases of growth and development.

If we could carry the experiments further by spectrophotometric, radiometric and other methods, including extraction of certain biological constitutents such as phospholipids, and by other chemical and biophysical studies I am sure a clearer picture of the effectiveness and quality of each individual pesticide would be obtained.

S.V. ANDREEV: Yes, I fully agree with you that at present specialists in pest control do not pay sufficient attention to the condition of the plant which is being protected. Many insecticides cause considerable physiological changes and damage to plant functions. In studying the influence of insecticides, therefore, it is necessary not only to study the effect on the insects but also on the plant itself, in order to be able to find the most appropriate methods and compounds.

H.J. BOROUGHS: Since it is known that injury to leaves may increase the absorption of viruses or nutrients, would you please explain how it is known that the increased absorption of phosphate in the presence of DDT is caused by interference with metabolism rather than just damage to the leaf substance?

S.V. ANDREEV: When chloro-organic insecticides are used against insect plant-pests in higher concentrations, the phosphorus uptake of the plant is damaged. With an increase in phosphate concentration, however, through the leaves, and not the roots, there is a sharp increase in phosphorus exchange, owing to the additional phosphorus. This considerably lowers the toxic effect of the chloro-organic insecticides which penetrate the plant and disrupt the course of its normal physiological processes. It is particularly important to apply these insecticides simultaneously under unfavourable conditions of plant growth. In our experiments the epidermis of the leaves was undamaged and the experiments were conducted only on normal plants.

E.E. TURTLE: I was interested in the reference to the use of a labelling technique to assess the amount of secondary damage to wild birds and mammals during the rodent control campaign. You used barium fluoracetate as your rodent control agent, but what did you use to label it?

S.V. ANDREEV: Yes, we used oats poisoned with barium fluoracetate and labelled with radioactive phosphorus. The presence of radioisotopes in the poisoned bait made it possible to determine accurately how much

poisoned bait was eaten by game animals and birds. If they died, it made it possible to determine the real causes, under field conditions, owing to the presence of radioactivity in the body.

We found what percentage of birds were taking the bait. By shooting them, we were able to establish that it was very small. We also found in certain cases that poisoned bait intended for rodents was in fact eaten by steppe antelopes, though the doses lethal for rodents were not lethal for antelope.

M. FRIED: I should like to ask three questions with regard to the grain disinfestation programme you mentioned.

Firstly, what is the efficiency of utilization of the radiation from the Co^{60} source and does all the grain receive the dose you referred to, i.e. 7000 r?

Secondly, can this method be applied to grain moving at 200 or more tons per hour, which is the present rate of movement in commercial plant operations?

Thirdly, have you any experience of using electronic accelerators to accomplish the same purpose?

S. V. ANDREEV: Yes, as I said, doses of 7 000 - 10 000 r ensure an overall activity of 60 kg Ra equiv.; to make sure of sterilizing all the insects we give 10 000 r. Uniformity of grain irradiation is obtained by stirring it up when it is moving forward, close to the rod source, by means of spiral planes inside the grain-pipe. The rod-type source ensures that the activity is used 100% because it is completely surrounded by moving grain. If we used a slot-type or a flat source, and the grain were to pass on one side of it only, we would have only 50% efficiency.

In reply to your second question, the gamma disinfestation plant is designed for a thoughput of 30 t/h. In general it operates on leguminous plants (peas, haricot beans, etc.) and is used to rid them of Acanthoscelides obtectus Say, Bruchus pisorum L. etc., though in principle the same kind of plant could be used to get rid of storage pests in grain. The principle remains the same. The capacity of the plant could be expanded by enlarging it, increasing the power source and lengthening the grain run.

As to the last question, electronic accelerators have not been used in our institute, though possibly they are being used in other institutes.

P. de PIETRI TONELLI: If I heard correctly, you said that an insecticide can influence the physiology of plants. Are you talking about phytotoxicity, cuticular phenomena, as you mentioned with regard to DDT and organo-phosphate absorption, or real internal physiological alterations? If so, may I have more details about this influence?

S. V. ANDREEV: In our work on the study of the effect of chloro-organic insecticides on physiological processes, we had in view mainly the effect produced by the penetration of toxic molecules on metabolism in plants, and in particular on phosphorus metabolism. We did not investigate the microscopic changes in the leaves, since the insecticide doses mentioned were within standard limits and a distinct change was observed at these amounts as well as inhibition of the uptake of phosphates by the plant from the soil.

C. H. SCHMIDT: You have told us that the possibility of using the sterile-male technique is being actively studied in your country. Have any

of these investigations reached the field stage and if so, with what insects?

S.V. ANDREEV: Research has begun on sterilization doses for such insect pests as Chloridea obsoleta F., Chortophila brassicae B., Leptinotarsa decemlineata and Pectinophora malvella Hb. We are also trying to determine the required ratio of sterilized individuals to the normal, as found in nature.

Research is also being carried out on polygamous and monogamous females and on the effectiveness of applying the method in these and other cases. In the forthcoming season it is intended to carry out a test of the sterilization technique and to use it against the Colorado potato beetle in its local habitat, i.e. in places where the potato fields are surrounded by woods or hilly country. Experiments will be conducted in isolated insectaries in field conditions in order to clarify the effect of various sterilization procedures on males and females. The fundamental difficulties of using this technique against agricultural pests include the difficulty of obtaining enough insects under artificial conditions and the limited number of generations obtainable from plant pests.

II.

LABELLED INSECTIDE STUDIES: TECHNIQUES

SOME APPLICATIONS OF RADIOISOTOPES TO THE STUDY OF THE CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS*

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Abstract — Résumé — Аннотация — Resumen

SOME APPLICATIONS OF RADIOISOTOPES TO THE STUDY OF THE CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS. Preliminary work was carried out using di-iodo-octadecane-I^{131} as a tracer in solution in oils. This substance proved useful for investigating the creep of oil films over insect epicuticle, but was unsatisfactory for critical work on oil absorption through the cuticle, being converted in the tissues to one or more water-soluble derivatives.

In subsequent investigations, dieldrin-C^{14} has been used in solution in oils labelled with tritiated hexadecane. In comparative experiments with Tribolium castaneum exposed to three solutions of different viscosity, appreciable differences in both the rates of diffusion over the insects and in absorption through the cuticle have been found. After an initial period, dieldrin is absorbed relatively faster than solvent, the magnitude of the differential absorption varying with viscosity.

QUELQUES APPLICATIONS DES RADIOISOTOPES DANS L'ÉTUDE DE LA CONTAMINATION DES INSECTES PAR DES INSECTICIDES EN SOLUTIONS. Les travaux préliminaires ont été exécutés en utilisant, comme indicateur, du bi-iodo-octadécane marqué par ^{131}I en solution huileuse. Cette substance s'est révélée utile pour l'étude de la dispersion des pellicules d'huile sur l'épicuticule des insectes, mais elle n'a pas donné de résultats satisfaisants lors de travaux sur l'absorption de l'huile à travers la cuticule du fait qu'elle se trouvait transformée dans les tissus en un ou plusieurs dérivés solubles dans l'eau.

Au cours d'études ultérieures, la dieldrine marquée par ^{14}C a été utilisée en solution huileuse marquée à l'aide d'hexadécane tritié. Des expériences comparatives sur le Tribolium castaneum, exposé à trois solutions de viscosités différentes, ont révélé des différences sensibles en ce qui concerne les vitesses de dispersion sur l'insecte et l'absorption à travers la cuticule. Après une période initiale, la dieldrine est absorbée à une vitesse relativement plus grande que le solvant, la différence étant fonction du degré de viscosité.

НЕКОТОРЫЕ ВИДЫ ПРИМЕНЕНИЯ РАДИОИЗОТОПОВ ДЛЯ ИЗУЧЕНИЯ ЗАГРЯЗНЕНИЯ НАСЕКОМЫХ РАСТВОРАМИ ИНСЕКТИЦИДОВ. Была выполнена предварительная работа с использованием дииодооктодекана, меченного J^{131}, в качестве индикатора в растворе в маслах. Это вещество оказалось удобным для исследования покрытия масляными пленками эпикутикулы насекомого, но было непригодным для критической работы по определению абсорбции масла через кутикулу, так как оно превращалось в тканях в одно или несколько водорастворимых производных.

В последующих исследованиях использовался меченный C^{14} дильдрин в растворе в маслах, меченных насыщенным тритием гексадеканом. В сравнительных опытах с использованием Tribolium castaneum в виде трех растворов различной вязкости была обнаружена заметная разница как в отношении скоростей диффузии у насекомых, так и в отношении абсорбции через кутикулу. После начального периода дильдрин абсорбировался быстрее, чем раствор, а величина дифференциального поглощения менялась в зависимости от вязкости раствора.

EMPLEO DE LOS RADIOISÓTOPOS PARA ESTUDIAR LOS EFECTOS CONTAMINADORES DE LAS SOLUCIONES INSECTICIDAS. Las primeras investigaciones se hicieron utilizando como marcador diyodo-octadecano-I^{131} en solución oleosa. Esta sustancia ha resultado útil para estudiar la digresión de las películas de

* This work was supported by the Tropical Pesticides Research Committee of the Department of Technical Co-operation of the United Kingdom Government.

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Au cours d'études ultérieures, la dieldrine marquée par ^{14}C a été utilisée en solution huileuse marquée à l'aide d'hexadécane tritié. Des expériences comparatives sur le Tribolium castaneum, exposé à trois solutions de viscosités différentes, ont révélé des différences sensibles en ce qui concerne les vitesses de dispersion sur l'insecte et l'absorption à travers la cuticule. Après une période initiale, la dieldrine est absorbée à une vitesse relativement plus grande que le solvant, la différence étant fonction du degré de viscosité.

НЕКОТОРЫЕ ВИДЫ ПРИМЕНЕНИЯ РАДИОИЗОТОПОВ ДЛЯ ИЗУЧЕНИЯ ЗАГРЯЗНЕНИЯ НАСЕКОМЫХ РАСТВОРАМИ ИНСЕКТИЦИДОВ. Была выполнена предварительная работа с использованием диодооктодекана, меченного J^{131}, в качестве индикатора в растворе в маслах. Это вещество оказалось удобным для исследования покрытия масляными пленками эпикутикулы насекомого, но было непригодным для критической работы по определению абсорбции масла через кутикулу, так как оно превращалось в тканях в одно или несколько водорастворимых производных.

В последующих исследованиях использовался меченный C^{14} дильдрин в растворе в маслах, меченных насыщенным тритием гексадеканом. В сравнительных опытах с использованием Tribolium castaneum в виде трех растворов различной вязкости была обнаружена заметная разница как в отношении скоростей диффузии у насекомых, так и в отношении абсорбции через кутикулу. После начального периода дильдрин абсорбировался быстрее, чем раствор, а величина дифференциального поглощения менялась в зависимости от вязкости раствора.

EMPLEO DE LOS RADIOISÓTOPOS PARA ESTUDIAR LOS EFECTOS CONTAMINADORES DE LAS SOLUCIONES INSECTICIDAS. Las primeras investigaciones se hicieron utilizando como marcador diyodo-octadecano-^{131}I en solución oleosa. Esta sustancia ha resultado útil para estudiar la digresión de las películas de

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aceite sobre la epicutícula de los insectos, pero es inadecuada para investigar la absorción de aceites por la cutícula, pues una vez en los tejidos se transforma en uno o más derivados solubles en agua.

En investigaciones consecutivas se empleó Dieldrin-^{14}C disuelto en aceites marcados con hexadecano tritiado. Comparando los experimentos realizados con Tribolium castaneum tratado con tres soluciones de viscosidad diferente se han podido observar diferencias apreciables en los índices de difusión en los insectos y de absorción cuticular. Después de un período inicial, la velocidad de absorción del Dieldrín es relativamente más rápida que la del disolvente; la diferencia depende de la viscosidad.

INTRODUCTION

When an oily solution of an insecticide is picked up by an insect, the oil facilitates the action of the insecticide in at least two ways: firstly, by spreading the insecticide over the epicuticle of the insect and thus presenting it very intimately to the insect; secondly, by facilitating the diffusion of the insecticide through the cuticle. These processes have not hitherto been studied quantitatively, and indeed it is difficult to see how they could be without the employment of radioactive tracer techniques, as no chemical method of analysis for small quantities of hydrocarbon oils is known.

Two series of experiments on this subject have been carried out. The first series was principally concerned with the spread of oil over the cuticle of active insects, for which purpose iodine-131 in the form of an iodized hydrocarbon was employed as a tracer. The second series was particularly concerned with the penetration of both oily solvent and an insecticide, dieldrin, through the cuticle. In this series the oils and insecticide were labelled with tritium and carbon-14 respectively.

1. EXPERIMENTS WITH DI-IODO-OCTADECANE AS AN OIL TRACER

Materials and methods

Traces of di-iodo-octadecane-I^{131}, prepared by the addition of iodine-131 to the unsaturated hydrocarbon octadecene, were added to a highly refined mineral oil, Shell Risella 17, which consists of hydrocarbons of chain length varying from 17 to 20 carbon atoms. The specific activity of the oil sample was adjusted to 2 mc/ml. This tracer was selected because of its suitability for liquid and solid counting techniques using Geiger-Müller tubes, the only methods of radio-assay available in the department at that time.

The oil deposits used in the experiments were always of 3 μl/cm^2 evenly deposited on Whatman No. 1 filter papers. Blowflies of the species Phormia terraenovae R-D were exposed to the oiled substrate at 25°C for varying periods of time up to 60 min, by a method described previously [1].

Immediately after removal from the active substrate, some of the flies were rapidly dissected to enable assays of oil associated with different parts of the body to be carried out. The films of oil adhering to the epicuticle of whole or part insects were washed off by three successive rinses of petroleum hydrocarbons, boiling range 40-60°C. This treatment was shown by control experiments with untreated flies to remove the epicuticular waxes. It is therefore clear that the adherent oil was totally removed, together with the fraction taken up in the epicuticular wax.

The rinsed insects were then ground up in anhydrous sodium sulphite and extracted, first in ether, then in 10% NaOH to obtain the absorbed tracer. Washings and extracts were assayed with a Geiger-Müller liquid counter tube. The ground residue was finally dried and checked for any residual activity with a thin-end-window Geiger-Müller counter.

Other individuals were dissected and mounted between 2 layers of thin adhesive tape weighing 8.06 mg/cm^2, and the preparations were exposed on X-ray film for one week. From the resulting autoradiographs, the location of oil on the body surface could be determined and its rate of spread gauged qualitatively.

Results

A brief summary of the more significant results only will be given, to illustrate the scope and limitations of this tracer substance in the study of oily films on insects. Some aspects have been partially described elsewhere [2].

Two processes were distinguished in the contamination of an insect on an oily deposit. Firstly, there is the primary uptake of oil by direct transfer from the substrate to the tarsi of the insect; secondly, there follows a diffusion of oil away from the tarsi up the legs to the general body surface.

Initially, oil was taken up by the tarsi at a mean rate of 3×10^{-7} ml/min per fly, the rate of uptake remaining substantially constant so long as the fly was actively walking. The oil diffused over the surface of the insect away from the tarsi at the rate of approximately 1.5×10^{-7} ml/min to all parts of the body.

The wings accumulated oil at 15×10^{-8} ml/min. Since the molecular surface area of the wings of this strain of the species is known, having been calculated by gas-adsorption methods [3], it is possible to calculate the mean thickness of oil film on the wing for any given treatment. It appears that if the oil molecules are randomly oriented on the surface, a monomolecular layer is established in 5 - 15 min from the initial contact of insect with deposit.

Autoradiographs confirm that the wing membranes are covered with tracer molecules after this period. They also reveal higher concentrations of radioactivity, indicating oil, along veins and at the confluence of wing veins. Similarly, they show uneven distribution in other parts of the body, especially the legs, the tarsi being, of course, more heavily contaminated than the upper segments.

If the oil were spreading by normal surface-tension forces, these differences would certainly be lost and an even film would be established quite soon after the removal of fly from deposit. But the differences persist without change, as successive autoradiographs show. This evidence, coupled with the fact that uptake varies with the activity of the fly, suggests that the oil proceeds over the cuticle aided by the very small elastic deformations of the cuticle which occur during active movements of an insect [4]. When the insect is killed, the movement of the very thin oil film ceases.

The oil, advancing at first as a mono-molecular layer, is simultaneously being absorbed. When we consider the absorption of oil, the limitations

of this tracer compound as a guide to oil movement become apparent, for the halogenated hydrocarbon tracer may well be differentially absorbed from the oil. But a second disadvantage also became apparent when measurements of the rate of absorption of tracer were made. Not all the I^{131} could be recovered in ether extractions; for example, after 60 min only 12.8% of the I^{131} was extracted in ether, 55.6% of the residual I^{131} was recovered with boiling water and 98% of the remainder was extracted with 10% NaOH solution. Thus it is clear that the I^{131} moiety of the tracer molecule is converted after absorption to one or more water-soluble compounds.

To sum up, di-iodo-octadecane-I^{131} has proved useful as a tracer for oil movements on the surface of an insect, but is not suitable as a tracer for oil movements across the cuticle or within the tissues.

2. EXPERIMENTS WITH DIELDRIN-C^{14} AND HEXADECANE-1:2-H^3

Materials and Techniques

Dieldrin-C^{14} of specific activity 6.54 mc/mM, obtained from the Radiochemical Centre, Amersham, was dissolved in three solvents of differing viscosity to form 1% solutions. The three solvents were n-hexadecane and the Shell oils Risella 17 and Risella 33, having viscosities of 2.9, 22.1 and 187 cP, and hydrocarbon chain lengths of 16, 17-20 and 20-35, respectively. The labelled hydrocarbon n-hexadecane-1:2-H^3 of specific activity 207 mc/mM was also added to each solution to allow the tracing of the hydrocarbon solvent molecules. The specific activity of the n-hexadecane-1:2-H^3 in each oil sample was adjusted to 27.5 mc/ml.

Filter-paper slips were treated as before to provide a deposit of 3 ml/cm^2 of solution, and batches of insects of the species Tribolium castaneum Herbst. were allowed to crawl over the deposits of 25°C for periods ranging from 30 min to 24 h.

After removal from the deposit, batches of ten insects were washed with three rinses of petroleum hydrocarbons (boiling range 40-60°C) to remove the adherent solution. The washed insects were then ground up with anhydrous sodium sulphite and extracted first with hexane for 2 h, then with water to recover absorbed oil, insecticide and any water-soluble metabolites of the insecticide which might be formed.

The quantities of carbon-14 insecticide and tritiated hydrocarbon in each sample were assayed and discriminated by means of internal liquid scintillator techniques. The scintillation liquids, obtained already formulated from Nuclear Enterprises (G.B.) Ltd., were NE213 based on xylene for the hexane extracts and external washes, and NE220 based on dioxane for the aqueous extracts. In the xylene-based scintillator, the counting efficiency was 6.5% for H^3 and 73% for C^{14}, under the conditions of test. In the dioxane-based scintillator, the maximum efficiency for C^{14} was rather lower, though it should be mentioned that the presence of relatively high concentrations of sodium sulphite caused the formation of a flocculant precipitate when the sample was added to the scintillator solution. This introduced much noise of a very low energy level, possibly due to chemiluminescence, which would have interfered with tritium assay, but could be discriminated from the C^{14} response.

Results

Adherent film of solution

The insects became contaminated more rapidly with the hexadecane solution than with the more viscous oil solutions, differences becoming apparent from the first measurement, taken after 30 min, when less than 1×10^{-8} g of dieldrin had been accumulated.

In contrast, at first no significant differences between the rates of contamination with Risella 17 and Risella 33 solutions were found. Even after many hours exposure, differences between the adherent quantities of these two solutions were relatively small.

The faster rate of uptake of hexadecane solution is maintained for about 16 h. At this time the insects are moribund or very feeble, and the rate of uptake of all three solutions falls to a much lower level (Table I). As

TABLE I

ADHERENT FILMS: QUANTITIES OF SOLVENT AND DIELDRIN RECOVERED FROM THE EXTERIOR OF TRIBOLIUM CASTANEUM AFTER EXPOSURE TO 3-μl/cm^2 DEPOSITS OF 1% SOLUTIONS ON FILTER PAPER

Duration of exposure (h)	Hexadecane solution	Risella 17 solution	Risella 33 solution
	SOLVENT ($\times 10^{-6}$ ml)		
0.5	0.92	0.28	0.34
1.0	1.37	0.61	0.47
2.0	2.28	0.83	1.02
4.0	2.37	0.85	1.08
8.0	3.61	2.15	1.75
16.0	9.0	2.52	2.33
24.0	9.31	3.19	2.56
	DIELDRIN ($\times 10^{-8}$ g)		
0.5	0.75	0.31	0.22
1.0	1.15	0.62	0.37
2.0	1.67	0.76	0.91
4.0	2.10	0.92	1.19
8.0	4.34	2.01	2.29
16.0	8.70	3.18	2.80
24.0	9.24	3.3	2.95

would be expected, the ratio of insecticide to solvent in the adherent films does not differ from that in the original solutions applied to the substrate.

Absorption of solvent and insecticide

The three solvents were absorbed through the cuticle at a rate of approximately 2×10^{-7} ml/h at first, becoming progressively slower. There were no appreciable differences between the rates of absorption of the three solvents (Fig. 1).

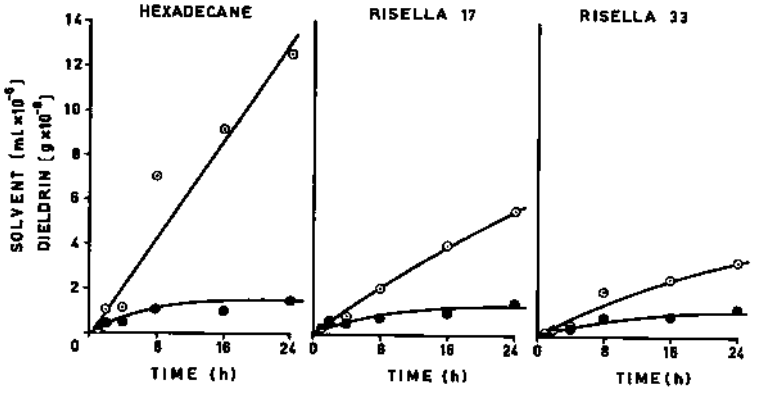

Fig. 1

Quantities of solvent and dieldrin extracted from the tissues of Tribolium castaneum Herbst. after exposure to 3-µl/cm² deposits of 1% solutions on filter paper

○ Dieldrin absorbed
● Solvent absorbed

However, the rates of absorption of dieldrin from the three solutions varied considerably. In the first hour, the average rate of uptake of dieldrin was relatively slower than that of the solvent. Subsequently, the insecticide was absorbed relatively faster than the solvent, the rate of uptake of dieldrin being approximately linear for each formulation (Fig. 1).

The magnitude of the differential absorption of dieldrin from each solution is illustrated by Table II, where the differential is expressed as the ratio (absorbed dieldrin over absorbed solvent) × 100. This ratio would be 1.0 for uniform absorption of both components of a 1% solution, but it will be seen that it rises steadily within 16 h to values of 8.36, 4.14 and 2.96 for solutions in hexadecane, Risella 17 and Risella 33 respectively.

Water-soluble metabolites

No evidence of water-soluble derivatives of dieldrin was found in the extracts.

TABLE II

DIFFERENTIAL ABSORPTION OF DIELDRIN FROM THREE 1% SOLUTIONS BY TRIBOLIUM CASTANEUM.

The differential is expressed as the ratio (absorbed dieldrin/absorbed oil) ×100

Treatment (h)	Hexadecane solution	Risella 17 solution	Risella 33 solution
0.5	1.09	0.25	0.63
1.0	0.88	0.79	0.82
2.0	2.87	1.16	1.01
4.0	2.74	1.65	1.40
8.0	6.45	2.81	2.43
16.0	8.36	4.14	2.96
24.0	8.46	3.96	2.74

Discussion

Although octadecane-di-iodide-I^{131} has certain limitations as a tracer for oil movements, significant results were obtained from its use; in particular, the importance of active movements of the cuticle in facilitating the uptake and spread of very thin oil films over an insect was demonstrated. However, the physical properties of the solvent also affect the rate of uptake, at least for films several molecules thick, for when crawling insects (Tribolium castaneum) were exposed to three solutions of different viscosity labelled with n-hexadecane-1:2-H^3, the least viscous was picked up appreciably faster than the more viscous (Table I).

The effects of solvent viscosity on the relative toxicities of different insecticide solutions has been noted previously from biological assays with Aëdes aegypti L. and Musca domestica L. [5]. The results of the present work permit the factors concerned to be analysed in greater detail.

The rates of absorption of the three solvents through the living cuticle were not significantly different, despite the differences in molecular size (Fig. 1). This result contrasts with earlier observations that light oils penetrate isolated pieces of cuticle faster than heavier oils [6]. The fact that no such differences were revealed in the present work suggests that the absorptive capacity of the hypodermal cells exercises a limiting effect on the rate of solvent absorption through living cuticle. The progressive decline in solvent absorption rate with time provides evidence of a saturation phenomenon.

The concentration of dieldrin in the film adhering to each insect and the rate of solvent absorption are the same for each formulation. Therefore, if the solvent facilitated the entry of dieldrin principally by carrying

the insecticide molecules in association with solvent molecules through the cuticular barriers, the quantities of dieldrin absorbed would be the same for each formulation.

But the quantities of dieldrin absorbed vary with solvent viscosity, and are much greater than would be expected on the basis of a direct solvent carrier effect (Fig. 1, Table II). Indeed, the capacity of the tissues to absorb dieldrin is considerable, and is not diminished by death, for the uptake of insecticide varied linearly with time and, unlike the solvent absorption, showed no appreciable indication of a saturation effect within a period of 24 h. Differential absorption of dieldrin from the adherent film was made good by diffusion from the substrate.

If neither concentration gradients nor direct carrier effects can explain the differences between the rates of dieldrin absorption from the different solvents, the facilitated absorption must depend almost entirely upon a modification of the cuticular barrier by the intercalation of solvent molecules, permitting faster transfusion of insecticide molecules than would otherwise occur. WIGGLESWORTH [6] has shown that the wax layer delays the absorption of pyrethrin solutions by Rhodnius nymphs. One likely effect of the oily solvents in the present experiment is to disturb the organization of the epicuticular wax. The smaller are the molecules of the solvent hydrocarbons dispersed in the wax layer, the faster the diffusion of dieldrin would be expected to take place.

In this context, it would be of interest to compare the effects of different solvents on insecticide absorption by a species having a structurally different type of epicuticle, e.g. blowfly larvae. Indeed, much remains to be done before the mechanisms of insecticide absorption are fully understood; it is certain that radioactive tracer techniques will be of great value in such studies.

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DISCUSSION

J. HALBERSTADT: The author reports no significant difference in the cuticle absorption rates as between hexadecane, Risella 17 and Risella 33, using tritiated hexadecane as tracer for all three solutions. But is he not in fact measuring the absorption of hexadecane in all three cases, so that there may be a difference of absorption between hexadecane and the two Risella oils? If that were the case, then there might be a much smaller difference in spreading rates between hexadecane and the two Risella oils,

which could be explained by assuming that the tracer disappears out of the Risella oil by absorption in the cuticle. That would convey the impression that the Risella oil spreads more slowly, while in fact the oil, now less active or inactive, might go on spreading at much the same rate as hexadecane.

C. T. LEWIS: The tracer must be giving a faithful measure of the absorption of the hexadecane solvent as it is chemically identical, and we are led to the conclusion that the facilitated absorption of dieldrin depends on a modification of the cuticular barriers and on this result alone; for much more dieldrin is absorbed than could be transported directly in the volume of solvent absorbed. The smaller absolute quantities of dieldrin-C^{14} absorbed from the Risella oils show that the cuticular barrier is less affected by the longer oil molecules, independently of the effect on the volumes of oil absorbed. Thus my principal conclusions still obtain.

If, as you suggest, the tracer is absorbed differentially from the Risella oils, the longer but otherwise similar Risella molecules must be penetrating more slowly than the tracer. In that event, the differential absorption of dieldrin relative to Risella oils would be greater than Table II suggests.

However, I still favour the view that the hexadecane-H^3 tracer moves proportionally with the oils in which it is dissolved, or very nearly so. That is certainly the case for movements of oil on the cuticle surface, to take your second point, which was that a differential absorption of H^3 tracer from the Risella oils would give an impression that the oils were spreading more slowly than the hexadecane solvent. That could be true only if the H^3 tracer was absorbed faster from the Risella oils than from the hexadecane; but the results of Fig. 1 show that this is not the case.

Moreover, if the totals of H^3 tracer, absorbed plus adherent, are added for the three solvents, it will be seen that the total for the hexadecane solvent taken up on the insect is much greater than the total for the Risella oils. Thus the H^3 tracer is not behaving independently of the oils in which it is dissolved.

D. A. CROSSLEY: I recall reading a paper published by Cumpel and Casida in 1957 on the metabolism of I^{131} by the cockroach Periplaneta, in which ingested I^{131} was found to be concentrated in the cuticle. If that is really a site of iodine concentration, it might explain some of your difficulties with I^{131}.

C. T. LEWIS: Yes, I think that the use of the iodized hydrocarbon for experiments on absorption is not acceptable.

F. T. PHILLIPS: I would like to ask the author whether any humidity effects could be observed. The evidence suggests that movement of the monolayer of oil is caused by small elastic deformations of the cuticle. Is this true for the wings, or could the spread of oil be facilitated or affected in any way by a presorbed layer of moisture? This could perhaps be shown in experiments with different humidities. Also, was it possible in your experiments that some of the oil was picked up on the wings by contact with the body?

C. T. LEWIS: Elastic stresses do occur in the wings, which possess several groups of campaniform sensilla which respond to such stresses. I have no information about the influence of adsorbed moisture on the creep of oil. It is possible that some of the oil passing to the folded wings may

have been transferred by incipient contact of the membranes proximally with the dorsal thorax, but the autoradiographs indicate the oil movement is principally by way of the articulations. If we assume oil was transferred by contact with the body, that still leaves unexplained the creep of oil over the whole of the wing. For that, one must still accept some suggestion of active cuticular movements which are on a sub-microscopic scale. Working with Dysdercus, which has much more rigid forewings than hindwings, I found that the spread occurs much more readily on the forewings than on the flimsy hindwings and this might very well be correlated with the elasticity of two types of wings.

K. van ASPEREN: Could the difference in absorption of dieldrin possibly depend on a difference of solubility in the oils used?

C. T. LEWIS: No; dieldrin has practically the same solubility in all three solvents used. Moreover Hadaway and Barlow (1958) found that the relative toxicities of solutions of DDT, and, I believe, of dieldrin also, did not vary with solubility in different solvents when topical applications were applied to Musca and Aëdes.

W. KLOFT: The ideal subject for this type of studies might be coccids (scales) of the type of Diaspididae, and this for two reasons. First, the females are more or less immobile, since they have no legs to allow screening movements and spreading of the insecticide over the cuticle in that way. Secondly, Diaspididae are well protected and covered by their scutum (scale) and must be controlled with oily solutions (or preparations in the vapour phase) since they suck in the plant's parenchyma and do not therefore pick up systemic insecticides. Your interesting method could be used to study how the oily film spreads from the scutum over the scale-body.

C. T. LEWIS: Your suggestion is very appropriate. A study of the uptake of insecticide from oily solutions and from the vapour phase by a scale insect is now in progress in my department. You mentioned that the oil could not be spread around by the legs. I would like to clear up any misunderstanding here. In the experiments on the creep of oil over the blow-fly, the insects were exposed only to oil and iodized tracer. There was nothing irritant in the solution and therefore no cleaning movements were performed. If a fly did perform random cleaning movements during the course of the experiment then it was noted and the results were not used for determining the rate of spread of oil, so that the spread that I refer to is not interfered with nor in any way assisted by the cleaning movements of an insect. Of course, with an insecticide solution in the field cleaning movements would be important.

J. R. OGLE: Like an earlier questioner, I would be inclined to doubt the validity of using hexadecane-H^3 to trace the rate of passage of a much heavier oil through the insect cuticle. Could you check this point by repeating the experiment with another tracer of rather higher or lower molecular weight? If the ratio of uptake of activity were then identical for the three oils, but different from those obtained with hexadecane, it would in fact suggest that you were measuring the rate of uptake of the tracer rather than the oil as a whole, and conversely, if the results were all in agreement, that would be confirmatory evidence that the nature of the solvent had little effect on its rate of uptake.

C. T. LEWIS: I would have liked to carry out such experiments. But a hydrocarbon of lower molecular weight than hexadecane would be too volatile for accurate work. No tritium-labelled paraffinic hydrocarbon of higher molecular weight is at present available, and the cost of synthesizing one would be considerable.

THE APPLICATION AND MEASUREMENT OF LABELLED RESIDUAL INSECTICIDES IN SOME PHYSICO-CHEMICAL STUDIES*

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Abstract — Résumé — Аннотация — Resumen

THE APPLICATION AND MEASUREMENT OF LABELLED RESIDUAL INSECTICIDES IN SOME PHYSICO-CHEMICAL STUDIES. The disappearance of residual films of insecticides from plant and other surfaces may be conveniently studied if the insecticide is labelled with a radioisotope of sufficient radiant energy to allow for a simple measuring technique.

Methods of application of insecticide solutions on different surfaces led to the design of a spray chamber suitable for the distribution of very small amounts (a few drops) of radioactive liquid formulations over a 35-cm² circular area.

Some measurements of the rates of volatilization of Cl^{36}-labelled dieldrin and aldrin crystals from glass surfaces are included.

APPLICATION ET MESURE DES INSECTICIDES RÉMANENTS MARQUÉS DANS CERTAINES ÉTUDES PHYSICO-CHIMIQUES. Pour étudier la disparition des pellicules que forment les insecticides rémanents sur les végétaux et d'autres surfaces, on a avantage à les marquer au moyen de radioisotopes ayant une énergie radiante suffisamment élevée pour pouvoir utiliser une méthode de mesure simple.

L'étude des méthodes d'applications des insecticides en solutions sur diverses surfaces a conduit à la mise au point d'un vaporisateur permettant de répartir de très faibles quantités (quelques gouttes) de liquide radioactif sur une surface circulaire de 35 cm².

Le mémoire donne les résultats de quelques mesures des vitesses de volatilisation de cristaux de dieldrine et d'aldrine marqués au ^{36}Cl déposés sur des surfaces de verre.

ПРИМЕНЕНИЕ И ИЗМЕРЕНИЕ МЕЧЕНЫХ ОСТАТОЧНЫХ ИНСЕКТИЦИДОВ ПРИ НЕКОТОРЫХ ФИЗИКО-ХИМИЧЕСКИХ ИССЛЕДОВАНИЯХ. Исчезновение остаточных пленок инсектицидов с растений и других поверхностей может быть легко изучено, если инсектициды мечены радиоизотопами с достаточной энергией излучения, позволяющей применять простые методы измерения.

В результате исследования методов нанесения растворов инсектицидов на различные поверхности была сконструирована камера, позволяющая распылять очень небольшие количества (несколько капель) радиоактивных жидкостей на круговую поверхность площадью 35 см².

Приводятся некоторые результаты измерений скоростей улетучивания со стеклянных поверхностей, меченных Cl^{36} кристаллов дильдрина и алдрина.

APLICACIÓN DE INSECTICIDAS MARCADOS Y MEDICIÓN DE SUS RESIDUOS EN ALGUNOS ESTUDIOS FISICOQUÍMICOS. La desaparición de las películas residuales de insecticidas que quedan en la superficie de las plantas y en otras superficies puede estudiarse fácilmente si los insecticidas se marcan con radioisótopos de una energía radiante que permita utilizar una técnica de medición sencilla.

Los métodos de aplicación de soluciones insecticidas sobre varias superficies han llevado al diseño de una cámara de pulverización capaz de distribuir cantidades sumamente pequeñas de solución (unas gotas) sobre una superficie circular de 35 cm².

La memoria reproduce los valores hallados para los índices de volatilización de cristales de Dieldrin y Aldrin marcados con ^{36}Cl, aplicados sobre superficies de vidrio.

* Work financed by a grant from the Tropical Pesticides Research Committee, Department of Technical Co-operation of the United Kingdom Government.

INTRODUCTION

In the study of the persistence of residual films of insecticides it is an obvious advantage to be able to follow the variation by a method which does not destroy or disturb the sample which is being measured. This may be done if a radiotracer is incorporated in the insecticide molecule, provided that this tracer truly follows the course of the molecule and does not exchange with similar atoms in different types of molecules and that further conditions are observed, namely, that the radioisotope has a half-life of sufficient duration to enable practical measurements to be made of the amount of radiation present, and that the type and strength of the radiant energy allows for a simple measuring technique. Thus, the chlorinated hydrocarbon residual insecticides may be conveniently studied if they are labelled with Cl^{36}, which has one of the longest half-lives known (3×10^5 yr) and whose β-radiation (0.714 MeV) can be measured simply and with high efficiency by means of a G-M end-window counter.

The laboratory application of thin and even films of insecticides on to a variety of surfaces may be accomplished in several ways with varying degrees of difficulty, but when the insecticides are radioactive (and in addition may have a high chemical toxicity to humans) either the difficulty of the method or the contamination hazards involved may preclude its use. For example, the sublimation of an insecticide from a warm to a cool surface [1], the controlled deposition of which would be especially difficult when using plant surfaces, or the spraying of the insecticide in an apparatus such as the Potter Tower [2], where special precautions would have to be taken, cannot be wholly satisfactory even under the best of conditions. The laboratory methods described in this paper involve a simple spreading method which has often been used but is suitable only for smooth surfaces, and a new spraying method which has been evolved for the distribution of a radioactive insecticide over a leaf surface or other plant surface.

THE VOLATILIZATION RATES OF PURE CRYSTALS OF DIELDRIN AND ALDRIN FROM GLASS SURFACE

Both the dieldrin and aldrin samples, containing Cl^{36} as radiotracer, were of specific activity 100 μc/g. The insecticide was dissolved in a suitable solvent (as described later) at a concentration of 4 mg/ml, and usually a 10 μl portion of this solution (containing 40 μg of insecticide) was spotted with a micro-pipette on to a 20-cm^2 circular area at the centre of a glass plate and spread evenly over this area with the edge of a thin glass coverslip. With experience, reproducible results giving an even coverage of 2 $\mu g/cm^2$ of insecticide crystals after evaporation of the solvent could be obtained by this method. Attempts to obtain an even coverage by wetting the glass surface with the pure solvent and then adding the radioactive solution dropwise, allowing it to spread by diffusion, usually resulted in an uneven coverage.

The crystalline insecticide film was measured radiometrically by placing the glass plate in a specially constructed Perspex castle containing an end-window G-M counter with a window area of 4.5 cm^2. The whole was

enclosed within lead shielding to reduce background counts to approximately 10 counts/min. The upper surface of the glass plate was 3 mm from the end-window, so that the central portion of the 20-cm^2 treated area was presented close to the end-window and a counting efficiency of over 20% was achieved. End-windows made of thin mica are often covered with a fine layer of graphite to eliminate photosensitivity. It is important to remove this layer (by wiping with cotton-wool moistened with alcohol), otherwise contamination of the counter-window by sorption of vapour from the radioactive insecticide will ensue.

Some results obtained for the rates of volatilization of these insecticides from glass surfaces are summarized below. The two ranges of crystal size were obtained by choosing a suitable solvent and allowing crystallization to proceed at room temperature (circa 20°C). "Cellosolve" (ethylene glycol monoethyl ether) gave large crystals and dioxan solvent gave small crystals.

(i) Dieldrin crystals, forming needles 1 - 10 mm long (average 5 mm long) at an initial deposit density of 2 $\mu g/cm^2$, gave a rate of volatilization of 0.06 - 0.08 $\mu g/cm^2 d$ at 20°C.
(ii) Dieldrin crystals, forming a milky-white deposit of needles <0.01-0.05 mm long (average, approx. 0.02 mm long) at initial deposit densities of 2 $\mu g/cm^2$ or 5 $\mu g/cm^2$, gave rates of volatilization of 0.12 - 0.14 $\mu g/cm^2/d$ at 20°C.
(iii) Aldrin, forming a milky-white deposit of rod-shaped crystals of average length approx. 0.01 mm at an initial deposit density of 2 $\mu g/cm^2$ lost most of this deposit at 20°C. after 1 d.

Thus, a reduction in the crystal size of dieldrin of the order of a hundredfold gave a twofold increase in the volatilization rate, although the volatilization rate was independent of the initial deposit density. Furthermore, the volatilization rates were linear, except for very low deposit densities. This shows that the rate of volatilization appears to be partly dependent on the surface area of the crystals forming the deposit, and also, in agreement with a similar observation of HOSKINS [3] to be independent of the initial weight per unit area of the deposit, except for very low deposit levels. The results also show the crystalline deposit of aldrin to be at least 15 times more volatile from glass surfaces than a similar deposit of dieldrin.

Volatilization rates of these crystalline deposits were sensitive to air movement and temperature change. To give extreme examples, whereas a 2 $\mu g/cm^2$ deposit of dieldrin crystals (1 - 10 mm long) at 20°C in still air took 3 - 4 weeks to fall to a deposit density of 0.2 $\mu g/cm^2$, the same tenfold fall was accomplished in 4 - 5 h at a temperature of 40°C and a wind speed of 2.0-2.5 mile/h.

It was noticed that although the volatilization rates of dieldrin and aldrin were linear, at very low deposit levels (circa 0.2 $\mu g/cm^2$) these rates decreased progressively, the curves of deposit density/time tending to approach the time axis asymptotically. This again is apparently in agreement with the observations of Hoskins.

THE APPLICATION OF SMALL AMOUNTS OF RESIDUAL RADIOACTIVE INSECTICIDES TO SURFACES BY A LABORATORY SPRAYING TECHNIQUE

The apparatus, shown in Fig. 1, consists of a glass tube of length 23 cm, internal diameter 6.6 cm and wall thickness 3 mm, with flat ground edges. The tube is gripped by nylon screws which allow for adjustment of its position, and the whole is supported by a stand. A brass plate, which bears the spray nozzle and 6 brass sorption tubes, is positioned on the top of the tube by a locating groove which is packed with a suitable grease (e.g.silicone high-vacuum grease) to form a seal. A brass platform at the bottom is fitted to a rack and pinion which moves the platform into the spraying position. A ball-and-socket joint which can be clamped in any position and joins the platform to the rack allows for adjustment of any tilt in the platform.

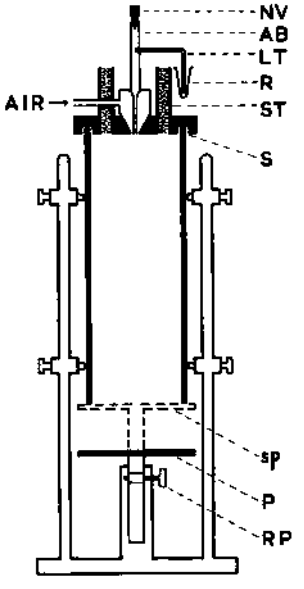

Fig.1

Apparatus for laboratory spraying technique

NV needle valve	AB air bleed	LT liquid tube
R reservoir	ST sorption tube	S seal
P platform	RP rack and pinion	sp spraying position

The atomizing nozzle consists of a brass body with an inlet for air under pressure and a 1.5-mm-diameter aperture into which a monel-metal liquid jet of external diameter 1.25 mm and internal diameter 0.75 mm fits concentrically. The tip of the liquid jet, which has a flat edge, is flush with the surrounding air orifice. A stainless-steel liquid tube of internal diameter 0.7 mm leads from the bottom of a small glass reservoir to the centre axis of a stainless-steel tube of internal diameter 1.75 mm. This latter tube allows an air bleed controlled by a needle valve to pass across the mouth of the liquid tube, and thus some atomization of the liquid occurs before it

reaches the main atomizing nozzle. This was found to be essential in order to prevent the formation of coarse liquid droplets in the spray chamber. The atomizing nozzle is inserted through a conical hole in the top brass plate and is clamped to the plate by screws which are also used for slight tilt adjustment in the nozzle. Silicone high-vacuum grease is again used to form an air-tight seal between the nozzle and brass plate. The six brass sorption tubes, each of 8-cm length and 8-mm internal diameter, slide into holes spaced equidistantly around the central atomizing nozzle and are soldered to the brass plate. They serve as exit tubes for the air during spraying and contain cotton-wool plugs which are used to trap any spray solution.

To obtain the optimum spraying conditions, the following procedure is adopted : a glass plate is placed on the platform so as to cover it completely and a filter-paper of somewhat larger diameter than the glass tube is placed on top of the plate. The platform is racked up firmly against the lower flat-ground edge of the tube so that the filter-paper forms a seal between the glass plate and the edge of the glass-tube. Tests have shown that no spray solution escapes past this seal. The air pressure to the atomizing nozzle is adjusted to 52 cm Hg (10 lb/in^2) by means of a mercury manometer in the air-line and cotton-wool plugs are inserted in the sorption tubes. The needle valve is adjusted so as to give an air-bleed flow-rate of 400 - 500 cm^3 air/min, adjustment being made at the same time in the resistance of the plugs to the air issuing from the spray chamber. This is achieved by teasing out or compressing the cotton-wool so that a water manometer records a positive pressure of 7 cm water inside the spray chamber or a suction pressure of 12 cm water in the liquid tube.

These spraying conditions are established quite easily, and spraying is quickly carried out by first racking down the platform, placing the leaf or other plant surface on the filter-paper (fixing with adhesive if necessary)and racking up again, and then presenting the reservoir containing the spray liquid to the liquid tube. As a precaution the apparatus, when in use, is placed in a well-vented fume chamber.

As is common with all spraying procedures, there were spray losses caused by air turbulence on the walls of the chamber and also entrainment of spray to the top of the chamber. Using both aqueous dye solutions and radioactive solutions of dieldrin in "Cellosolve", it was shown that the cotton-wool plugs in the sorption tubes were completely successful in holding back entrained spray solution. When water or "Cellosolve" was sprayed in the apparatus, approximately 15% of the spray solution showed a very fine droplet size and good coverage was obtained over the 35-cm^2 circular area at the bottom of the chamber. When small glass discs were placed on the platform and sprayed with radioactive solutions of dieldrin in "Cellosolve" and the crystalline deposits were assayed radiometrically, it was found that the central circular area of 5 cm^2 received an even coverage of the insecticide. The mean deposit density of the surrounding area was found to be slightly more than half that of the central area. The crystal sizes of the dieldrin deposits were in the range 0.1 - 1.5 mm (average 0.3 mm long), somewhat smaller than those obtained when similar solutions were sprayed in the Potter Tower (range 0.1 - 1.5 mm, average 1 mm long).

This apparatus has been developed for spraying small amounts of liquid; reproducible results have been obtained with as little as 0.05 ml liquid in the reservoir.

ACKNOWLEDGEMENTS

I wish to thank Dr. C. Potter, Head of the Department of Insecticides & Fungicides, for helpful advice and Mr. A.J. Arnold of the same Department for valuable technical help and the manufacture of the nozzle.

The samples of radioactive dieldrin and aldrin were a gift from Shell Research Ltd.

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DISCUSSION

G. F. BURNETT: Your deposit on the spray chamber is described as about 15% of that expended. How reproducible was this?

F. T. PHILLIPS: For five consecutive sprayings the amount of spray solution deposited ranged from $13\frac{1}{2}$ to $16\frac{1}{2}$% approximately (the approximate mean being 15%).

G. F. BURNETT: Did the deposit increase with repeated sprayings in the chamber?

F. T. PHILLIPS: Except for the initial spraying, which usually appeared to be less, there was no visible increase in the amount of spray deposited when 0.05 ml aqueous dye solutions were sprayed consecutively on to consecutive filter-papers until eventually run-off of spray solution occurred down the inside of the chamber.

D. F HEATH: Did you analyse the evaporation rates from glass slides in terms of the formula: rate = evaporation area × wind-speed, the effect of the temperature being exponential as usual? Your results seem on the whole to indicate that a formula of this sort might apply.

F. T. PHILLIPS: No, I have not used this or any similar formula showing correlations with wind-speed, because at the moment insufficient data have been obtained using different wind-speeds. Experiments using a range of wind-speeds are, however, to be conducted in the near future.

D. F. HEATH: What I had in mind was that you might have analysed your curve by determining, for example, the surface areas of crystals under the microscope.

F. T. PHILLIPS: No, I have not really done a proper analysis, though I can say that under the microscope at the very beginning, at a high deposit density, the crystals tend to be all joined together like fern-trees in arrangement. They touch at certain points. As time goes on, in a few weeks perhaps, these slowly disappear, until you get a very dispersed crystal system. The crystals themselves diminish in size. I did not measure the

diameters of the crystals. They are long needle crystals; all I measured was the length, which diminished with time. Finally they were not visible at all. After several weeks I still got a very low activity from the plates, although no crystals were observable in the microscope. The background was 10 counts/min in my operation, and I was getting counts of between 10 and 15 for long periods afterwards. So whether that was actually a radioactive count-out, or a slight contamination, I don't know. Certainly after five or six weeks, at any rate, one could say that the curves had reached the maximum and that practically no further radioactivity was observable.

J. W. MILES: Were evaporation rates measured on any surfaces other than glass?

F. T. PHILLIPS: No. Experiments using leaf or other plant surfaces were held up because of lack of a suitable method of applying insecticide solutions to these surfaces. With the design of the spray chamber described in this paper I hope now to go ahead with this work.

G. F. BURNETT: It is relevant, I think, to Mr. Miles' remarks that regular work is being done by HADAWAY and BARLOW on the disappearance of spread on mud surfaces*. They have obtained much bigger discrepancies in the rate of disappearance of crystals of different sizes than you have at Rothamsted. They graded the crystals into 1 μm, 10 μm and, I think, 100 μm. There the vapour phase disappears into the mud, which suggests that the removal of the vapour is extremely important in determining the rate of volatilization. You may find that if you increase your wind-speed above two miles an hour and completely remove your extracted air from contact with your surfaces, you considerably increase your rates of volatilization.

F. T. PHILLIPS: That is certainly true. At the moment I have only used two rates of air movement but, as you say, the evaporation rate increases quite remarkably with the rate of change of air movement.

Again, when we place the radioactive plates in still air, we have to be careful that although we shield the plates from draughts, we do not cover them: otherwise the concentration of vapour builds up in a covered container. Although there is some egress into the atmosphere, there is a considerable concentration above the plates and this diminishes the rate of volatilization. We have to find a happy medium between the two.

The suggestion that increased wind-speed might increase not only the evaporation rate but also the difference in evaporation rates due to different crystal sizes is a most interesting one. It seems also to agree with the formula cited by Dr. Heath and I shall certainly put it to the test when I come to do the work on variations in wind-speed.

K. von ASPEREN: In our own and other laboratories evidence has been obtained that chemical changes may occur under normal atmospheric conditions. Did you consider or exclude this possibility?

F T. PHILLIPS: These possibilities were considered, Any degradation by ultraviolet light could be excluded because of the conditions of the experiments; the glass plates with the insecticide deposits were held in a dark constant-temperature room at 20°C. Any oxidation of aldrin to dieldrin would probably be shown by a change in the rate of volatilization

as the experiment proceeded. This did not occur, except for the progressive decrease in rates at very low deposit levels which was noted for both aldrin and dieldrin. Also, most of the aldrin in this experiment volatilized during a short time (one day). Thus it was considered that no measurable amounts of aldrin oxidized to dieldrin.

K. von ASPEREN: I believe that other conversions could occur, but even then the occurrence of straight lines must be an indication that there is no chemical change in your experiment; is that right?

F. T. PHILLIPS: Not necessarily, but I do not think that there could be any other chemical changes except, as I said, a very slight change of aldrin into dieldrin. These are very stable compounds compared with most insecticides - that us why they are residual insecticides. The only strong instability is the fact that aldrine oxidizes to dieldrin, but that occurs generally when we spray into soil or any organic material; on glass plates there is very little opportunity for oxidation to take place.

RADIOAUTOGRAPHY IN THE STUDY OF RADIOISOTOPICALLY-TAGGED SUBSTANCES IN INSECT CONTROL

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Abstract — Résumé — Аннотация — Resumen

RADIOAUTOGRAPHY IN THE STUDY OF RADIOISOTOPICALLY TAGGED SUBSTANCES IN INSECT CONTROL. In return for a relatively small expenditure of effort, the newer, simplified techniques of radioautography offer great sensitivity and precision at the microscopic level in many phases of the investigations of singly or doubly radioisotopically-tagged insecticides: e.g., uptake, translocation, metabolism and determination of residues in insects, plants and animals. The physiology and biochemistry of irradiated insects are other phases which can be studied by radioautography. Radiation effects on the generative organs, especially, are being studied with tritiated thymidine. Whereas counting techniques yield gross information on the presence and quantity of radioisotopes, radioautography can provide information on actual sites of deposition in tissues or even parts of cells. This resolution is available on ordinary permanent histological preparations, in contrast to counting techniques in which accuracy of counting demands the destruction of the sample. Standard radioautography is especially useful for conveniently following fractional microcurie amounts of very weak emitters such as radiocarbon (C^{14}) and tritium (H^3), as opposed to the very expensive, highly sophisticated apparatus which is required for counting techniques when very weak emitters are employed.

The technique places no restriction on the isotope used for labelling, since even the radiation from tritium and C^{14} will register on the nuclear emulsion. In fact, tritium and C^{14} (which are desirable since nearly every insecticide will contain carbon and hydrogen) have the advantage of yielding high-resolution radioautograms and have long half-lives, so that if very long exposure times are necessary, there would be no difficulty. Long exposure times may be required since only tiny amounts of radioactivity, even of high-specific-activity insecticides, can be expected to be taken up. It is necessary to be certain that the insecticides are tagged at such a point in the molecule that the radioactive tag remains bound to the moiety which it is desired to study. Once the insecticide has been taken up or administered, the specimen preparation follows standard histological techniques of fixation, embedding, cutting and attaching to glass slides. The tissues can then be deparaffinized and hydrated and are then ready for application of the nuclear emulsion. If the radioactive compounds in the tissues are in a soluble state, freeze-drying techniques can be used to prevent loss or mobilization of the radioisotope. Dry-emulsion can be used for these preparations.

Staining can be done either before or after application of the nuclear emulsion, depending on the nature of the stain desired. In general, it is preferable to stain after the emulsion has been applied and photographically processed. With sufficiently energetic isotopes, whole mounts of plants or plant parts can be radioautographed, in which case histological preparation can be eliminated; the part to be radioautographed is simply compressed with the film in a plant press or other suitable device.

The most convenient method of applying nuclear emulsions to histological preparations is to dip them into melted bulk emulsion in the dark and then clean the bottom of the slide. The slides are then placed in a dark chamber, in a dry, inert atmosphere and allowed to expose for a time which must be empirically determined. At the expiration of this period, the slides are photographically processed and thoroughly washed and coverslips are mounted. The slides are then ready for study just as regular histological specimens at all magnifications. A slight upward adjustment of the focus brings into view the radioautographic pattern which can be thus easily studied in relation to the tissue. The recommended techniques and the simple, generally available laboratory equipment required are described.

L'AUTORADIOGRAPHIE DANS L'ÉTUDE DE SUBSTANCES MARQUÉES AU MOYEN DE RADIOISOTOPES UTILISÉES DANS LA LUTTE CONTRE LES INSECTES. Les nouvelles techniques simplifiées d'autoradiographie — d'une application relativement facile — sont très sensibles et précises au niveau microscopique dans de nombreuses phases des recherches sur les insecticides marqués au moyen de un ou deux radioindicateurs: recherches

sur l'absorption, la translocation, le métabolisme et les résidus chez les insectes, les végétaux et les animaux. L'autoradiographie peut aussi servir à étudier la physiologie et la biochimie d'insectes irradiés. On étudie notamment les effets des rayonnements sur les organes reproducteurs au moyen de thymidine tritiée. Alors que les méthodes de comptage fournissent des renseignements approximatifs sur la présence et la quantité de radioisotopes, l'autoradiographie permet d'obtenir des données sur l'emplacement exact du dépôt dans les tissus, voire dans des parties de cellules. Cette détermination peut se faire sur des préparations histologiques normales permanentes, alors que les méthodes de comptage impliquent la destruction de l'échantillon pour obtenir des résultats exacts. L'autoradiographie courante est particulièrement utile pour suivre des quantités de l'ordre d'une fraction de microcurie d'émetteurs très faibles tels que le radiocarbone (^{14}C) et le tritium (^{3}H) ; le comptage des mêmes émetteurs exige des appareils très coûteux et hautement perfectionnés.

L'autoradiographie n'exclut l'usage d'aucun radioisotope pour le marquage puisque même les rayonnements de ^{3}H et de ^{14}C impressionnent l'émulsion nucléaire. En fait, ^{3}H et ^{14}C (qui sont particulièrement intéressants du fait que tous les insecticides contiennent du carbone et de l'hydrogène) ont l'avantage de donner des autoradiogrammes à haute résolution et d'avoir de longues périodes; il s'ensuit qu'il n'y a pas de difficultés lorsqu'un très long temps d'exposition est nécessaire. C'est souvent le cas, parce que même pour des insecticides ayant une forte activité spécifique, les quantités de radioactivité que l'on peut espérer absorber sont généralement très faibles. Il faut veiller à ce que l'insecticide soit marqué en un point de la molécule tel que le radio-indicateur demeure lié à la portion que l'on désire étudier. Une fois que l'insecticide a été absorbé ou administré, on prépare les échantillons en appliquant les méthodes histologiques courantes: fixation, enrobage, découpage et pose sur lamelle de verre. Les tissus sont ensuite déparaffinés et hydratés; ils sont alors prêts pour l'application de l'émulsion nucléaire. Si les composés radioactifs se trouvent à l'état soluble dans les tissus, on peut faire appel aux méthodes de dessication par congélation pour éviter toute perte ou tout mouvement du radioisotope. Des films à émulsion sèche peuvent être utilisés pour ces préparations.

La coloration peut être faite avant ou après application de l'émulsion nucléaire, selon la nature de la couleur désirée. Il est préférable, en général, que la coloration intervienne après l'application et le traitement photographique de l'émulsion. Si l'on utilise des radioisotopes ayant une énergie suffisante, on peut procéder à l'autoradiographie de plantes entières ou de parties de plantes, et supprimer ainsi la préparation histologique; la partie à autoradiographier est simplement comprimée avec le film dans une presse ou dans tout autre appareil approprié.

La méthode la plus pratique pour appliquer les émulsions nucléaires aux préparations histologiques consiste à plonger l'échantillon dans une émulsion fondue et à nettoyer ensuite la partie inférieure de la lamelle. Les lamelles sont ensuite placées en chambre noire, dans une atmosphère sèche et inerte, et exposées pendant une durée qui doit être déterminée empiriquement. Après quoi, elles font l'objet d'un traitement photographique, sont soigneusement lavées et revêtues d'une protection. On peut alors les étudier comme des échantillons histologiques normaux, avec le grossissement voulu. Un léger déplacement du foyer vers le haut permet de voir l'autoradiographie, que l'on peut ainsi facilement étudier en regard du tissu. Le mémoire décrit en détail les méthodes recommandées ainsi que le matériel nécessaire, qui est simple et dont disposent généralement les laboratoires.

ПРИМЕНЕНИЕ РАДИОАВТОГРАФИИ ПРИ ИЗУЧЕНИИ СУБСТАНЦИЙ, МЕЧЕННЫХ РАДИОИЗОТОПАМИ, В БОРЬБЕ С НАСЕКОМЫМИ. При относительно небольших затратах труда более новые, упрощенные методы радиоавтографии обеспечивают большую чувствительность и точность на микроскопическом уровне во многих фазах исследований однократно и двукратно меченных радиоизотопами инсектицидов, например при исследовании поглощения, перемещения, метаболизма и определении остатков инсектицидов в растениях, у насекомых и животных. Физиология и биохимия облученных насекомых являются другими областями применения радиоавтографии. Воздействие облучения, особенно на генеративные органы, изучается с помощью меченного тритием тимидина. В то время как методы счета дают много информации о наличии и количестве радиоизотопов, с помощью радиоавтографии можно получить данные о фактических местах их отложения в тканях или даже в частях клеток. Получение такой разрешающей способности возможно на обычных постоянных гистологических препаратах, в то время как при применении методов счета для получения точного результата требуется разрушение образца. Стандартная радиоавтография особенно удобна для прослеживания малых количеств изотопов, составляющих доли микрокюри, очень слабых излучателей, таких, как радиоактивный углерод (C^{14}) и тритий (H^{3}), тогда как для работ с очень слабыми излучателями с применением методов счета требуются очень дорогие и сложные приборы.

Метод не имеет ограничений в отношении изотопов, применяемых для мечения, так как даже излучения трития и C^{14} будут фиксироваться на ядерной фотоэмульсии. Действительно, тритий и C^{14} (которые желательны, поскольку почти каждый инсектицид будет содержать углерод и водород) имеют то преимущество, что дают радиоавтограммы с высокой разрешающей способностью и имеют большой период полураспада и поэтому не возникает трудностей в тех случаях, когда необходим очень длительный срок экспозиции. Продолжительная экспозиция может потребоваться, когда предполагается наличие очень небольших количеств радиоактивных веществ даже при высокой удельной активности инсектицида. Необходимо быть уверенным в том, что инсектицид попадает в ту часть молекулы, где радиоактивный индикатор остается связанным с той половиной, которую желательно изучить. После того как инсектицид поглощен или введен, подготовка препарата осуществляется обычными гистологическими методами фиксации, заливки, приготовления срезов и наложения на предметное стекло. Затем ткани могут быть депарафинированы и гидратированы, после чего они готовы для покрытия ядерной фотоэмульсией. Если радиоактивные составляющие в тканях находятся в растворимом состоянии, то для избежания потери или мобилизации радиоизотопа могут быть применены методы сушки замораживанием. Для таких препаратов можно применять сухие эмульсионные пленки.

Окраска может производиться как до, так и после применения ядерной фотоэмульсии, в зависимости от характера требующейся окраски. В общем, предпочтительнее окрашивать после того, как фотоэмульсия нанесена и проявлена. При применении изотопов с достаточной энергией излучения радиоавтографированию могут подвергнуться растения или их части целиком, и в этом случае гистологическая обработка может быть исключена; та часть, которая должна подвергнуться радиоавтографии, прижимается к пленке в прессе для растительных образцов или в другом, пригодном для этого устройстве.

Наиболее удобным методом применения ядерных фотоэмульсий для гистологических препаратов является погружение их в расплавленную массу фотоэмульсии в темноте и затем очистка нижней поверхности предметного стекла. После этого предметные стекла помещаются в темную камеру с сухой инертной атмосферой и выдерживаются там в течение времени, определяемого эмпирически. Затем предметные стекла проявляются, тщательно промываются, и на них надеваются защитные стекла. После этого предметные стекла готовы для исследования, точно так же, как обычные гистологические препараты при всех увеличениях. Незначительное смещение фокуса вверх вводит в поле зрения радиоавтографический образец, который может быть таким образом легко изучен при сравнении с тканью. Дается подробное описание методов и необходимого лабораторного оборудования.

LA AUTORRADIOGRAFÍA EN EL ESTUDIO DE LAS SUSTANCIAS MARCADAS CON RADIOISÓTOPOS UTILIZADAS EN LA LUCHA CONTRA LOS INSECTOS. Las nuevas técnicas autorradiográficas simplificadas requieren cierto esfuerzo pero permiten obtener una gran sensibilidad y exactitud al nivel microscópico en el estudio de los insecticidas marcados con uno o dos radioisótopos, y, en particular, en el de la absorción, traslocación, metabolismo y determinación de residuos en plantas, insectos y otros animales. Con ellas se pueden estudiar también la fisiología y la bioquímica de los insectos irradiados. Se están investigando con timidina tritiada los efectos de las radiaciones sobre los órganos reproductores. Las técnicas de recuento dan información general sobre la presencia y la cantidad de los radioisótopos, mientras que la autorradiografía proporciona datos sobre el lugar exacto de fijación en los tejidos e incluso en partes de la célula. Este poder de resolución se alcanza con preparados histológicos ordinarios que pueden conservarse, contrariamente a lo que sucede con las técnicas de recuento que exigen, si se quiere lograr mayor exactitud, la destrucción de la muestra. La autorradiografía corriente es particularmente útil para analizar por métodos muy sencillos fracciones de microcurie de emisores muy débiles como el radiocarbono (^{14}C) y el tritio (^{3}H), mientras que las técnicas de recuento requieren en estos casos un instrumental muy complicado y costoso.

Esta técnica no impone limitación alguna en cuanto a los isótopos que pueden emplearse como indicadores, porque la emulsión nuclear registra incluso las radiaciones del tritio y del ^{14}C. En realidad, el tritio y el ^{14}C (que son convenientes puesto que casi todos los insecticidas contienen carbono o hidrógeno) tienen la ventaja de dar autorradiografías de elevado poder de resolución y, como son de período largo, permiten tiempos de exposición muy prolongados. Estos tiempos de exposición pueden resultar necesarios debido a las pequeñas cantidades de radiactividad que se absorben incluso empleando insecticidas de elevada actividad específica. Es preciso cerciorarse de que el insecticida esté marcado en un punto tal de la molécula que el átomo trazador permanezca ligado a la parte que se desea estudiar. Una vez absorbido o administrado el insecticida, la preparación de las muestras se efectúa siguiendo las técnicas histológicas corrientes de fijación, inclusión, microtomía, y colocación sobre portaobjetos. Seguidamente se elimina la parafina y se hidratan los

tejidos, que quedan así preparados para la aplicación de la emulsión nuclear. Si los compuestos radiactivos del tejido se encuentran en estado soluble, se pueden emplear técnicas de liofilización para evitar las pérdidas y el desplazamiento de los radioisótopos. Para estos preparados pueden utilizarse películas de emulsión seca.

El tinte puede efectuarse antes o después de aplicar la emulsión nuclear, según el tipo de coloración que se desee obtener. En general es preferible efectuar la operación después de haber aplicado y revelado la emulsión. Si se emplean isótopos de energía suficiente, es posible autorradiografiar plantas enteras o partes de plantas, en cuyo caso se evita la preparación histológica; la parte que se desee autorradiografiar se comprime con la película en una prensa o en otro dispositivo adecuado.

El método más conveniente para aplicar las emulsiones nucleares a los preparados histológicos consiste en introducirlas en la emulsión fundida y limpiar después el reverso del portaobjetos; evidentemente esta operación tiene que efectuarse en la oscuridad. Los preparados se colocan en una cámara oscura, de atmósfera seca e inerte, y se exponen durante un tiempo que se determina empíricamente. Transcurrido este tiempo, la emulsión se revela, se lava cuidadosamente y se colocan los cubreobjetos. Los portaobjetos pueden entonces estudiarse en cualquier ampliación, como los preparados histológicos corrientes. Un ligero desplazamiento ascendente del foco pone de relieve los detalles de la autorradiografía que, de este modo, se puede estudiar en relación con el tejido. En la memoria se describen en detalle las técnicas recomendadas y el sencillo equipo necesario, que suele encontrarse en todos los laboratorios.

INTRODUCTION

Participants in previous symposia [1, 2] which were closely related to this one in subject matter have repeatedly mentioned the usefulness of radioautography in entomological research. In spite of this, examination of publications reveals that only a few investigators are taking advantage of this tool. Perhaps this is due to the fact that in the past radioautography had been difficult to perform and often yielded equivocal results. Recent development of more sensitive and dependable emulsions, which are commercially available, and simpler techniques and apparatus now place radioautography in the category of a routine tool which should be considered in any research problem where the fate of tracers in an organism is of interest. This is especially true if the research is to be done in the field or in areas remote from the amenities of highly-refined counting equipment and the repair personnel and other support items which such equipment requires. Furthermore, the amounts of radioactivity required are smaller than for other procedures, and so handling the tagged compounds can be more convenient.

Even in laboratories where all the refinements are available, radioautography offers a type of information which cannot be obtained in any other way. While it is now possible to detect extraordinarily small quantities of even tritium and radiocarbon by liquid scintillation counting, only radioautography makes it possible to visualize the sites of deposition within a tissue (or with tritium-tagging, within cells) of a radioisotopically-tagged compound. The purpose of this communication is to provide general background information necessary for using radioautography.

Within the area of the special interests of those attending this Symposium, radioautography is applicable in many ways. It is ideally suited to studies of the uptake, translocation and metabolism of tagged insecticides in insects, animals and plants; in studies of the physiology and biochemistry of insects under normal conditions and following radiation; the response of the reproductive processes in insects in response to radiation; the transport of

radioactive parasites from host to host through the insect vector; location of radioactive parasites in the tissues of the host, including insect parasites such as Empusa muscae; sites of deposition in studies of the influence on resistance of radioactive trace elements in plants; movements of ions across membranes; and many others.

SELECTION OF THE RADIOISOTOPE TO BE USED

Radioautography itself does not place any restrictions on the radioisotope to be used, since the nuclear emulsions now available respond to even the very low energies emanating from tritium, the weakest of the radioisotopes. The decision as to which isotope will be used then is made on the basis of the physiological, biochemical and metabolic factors under study and the chemical constituents of the compound it is desired to investigate. The effective half-life and the mean path length in tissue and emulsion must also be considered, as well as type of radiation – alpha, beta or gamma – and degree of avidity of the organism for the labelled compound. The radiation dose delivered to the organism should be kept as low as possible. The possibility of an isotope effect where the mass of the radioisotope is very different from that of the non-radioactive sister, as in the case of tritium, which has three times the mass of hydrogen, must also be taken into account [3].

LABELLING

Insects have been labelled in many different ways. They have been exposed to radioactive gas, to artificial nutrients contaminated with radioisotopes, dipped into, injected, perfused, sprayed or painted with radioactive solutions, or allowed to feed on their natural food which has been made radioactive. Radioactive insecticides can be produced by substituting a radioactive isotope in the original synthesis [4], or by chemical manipulation of the insecticide, or by exchange, as with tritium [3].

In using a labelled insecticide, there are several factors to consider: half-life, decay products produced, isotopic effects, position in the molecule, specific activity attainable, radiochemical purity, radiochemical stability and the radiation dose to the animal. The half-life is important because when the radioisotope decays to a daughter product the chemical composition changes, and the insecticide may change its properties or the insecticide will be metabolized differently, or may become undetectable if the daughter product is not radioactive. The position on the molecule should be known, since the metabolism of the insecticide may break up the molecule and it may be necessary to know which moiety of the original molecule is being followed. In such a case, the insecticide per se may disappear, but the toxic metabolite may persist for weeks, and be demonstrated by the radioautogram. Reversible or irreversible exchange may also occur, particularly with tritium or radiocarbon. If the insecticide is not radiostable, the analysis of results will be confused because the original material will have changed. Very high specific activities are ordinarily desirable

unless they significantly reduce radiostability and/or deliver radiation doses to the insect sufficient to cause undesired radiation effects. Radiostability can sometimes be preserved by storing the insecticide in a solvent which helps absorb some of the radiant energy.

HISTOLOGICAL PROCESSING

The method of administration of the radioisotope or of the tagged insecticide will be determined by the experimental work which is being done and the kind of information being sought, so little can be said about this. However, the time allowed between administration of the isotope and fixation of tissue for radioautography and the time spent in histological processing must be kept as short as possible relative to the half-life of the radioisotope. Except for reagents which dissolve the radioisotope-bearing compounds out of the tissues, ordinary fixatives and embedding techniques can be used. Alternative techniques can be found if necessary; for instance, if the compounds of interest are soluble in organic solvents but not in water, one can use water-soluble carbowax instead of paraffin or celloidin for embedding [5]. If the compounds are water-soluble, freeze-dry techniques may be employed.

The thickness at which the sections are cut is determined by the cytological resolution required. Of course, thin sections will contain less isotope than thicker ones, but then there are problems with absorption within the specimen, so, in general, one simply cuts at the same thickness as for non-radioautographic work.

Since the stains used may also dissolve the radioisotope-bearing compounds and since some stains are decolorized by the photographic processing which the radioautogram must undergo, it is generally preferable to stain after the photographic processing. However, there is considerable leeway in selecting stains. In our laboratory, we have found [6] that some stains, such as Feulgen-fast green, chromium hematoxylin phloxine and aldehyde fuchsin-PAS, work best when applied before application of the nuclear emulsion. Others, such as celestin blue-Mayer's haemalum, metanil yellow-iron hematoxylin, lithium carmine-picric acid, Weigert acid-iron haematoxylin, alum cochineal, methyl green pyronin, indigo carmine-picric acid, methylene blue-azure A, toluidine blue, Nissl and Cason, are best when applied after the photographic processing. It was also found that some combination stains, such as trichrome-PAS, luxol-fast blue-PAS, haematoxylin-eosin and aniline blue-carbol fuchsin, worked best when part of the staining was performed before application of the nuclear emulsion and the remainder applied after the photographic processing.

NUCLEAR EMULSIONS

Photographic emulsions intended for use in radioautography contain up to 95% silver halide, mostly bromide, by weight, with an average grain size of 0.2 to 0.3 μm and a maximum of 0.5 μm. X-ray film has much larger grain size, up to 6 μm, which yields high sensitivity but poor resolution.

The response of the emulsion to radiation depends upon the characteristics of the radiation traversing it. Alpha particles, which have a large mass and move relatively slowly, give up their energy to atoms in or near their path but do not tend to be deflected from an approximately straight course. Therefore, passage of an alpha particle through the emulsion results in a fairly straight line defined by a series of silver granules further apart at the origin than at the end of the path. The track may actually consist of a cylinder composed of granules if the local ionization is sufficient in quantity. The track of a beta particle is much more complicated, since it has little mass and is easily deflected by atoms in its path. The beta track is tortuous and the individual granules composing it are relatively far apart. It is impossible to unravel a group of beta tracks if they pass through a small volume. X or gamma rays, which are very fast and of negligible mass, register in nuclear emulsion by eliciting secondary electrons, which yield tracks similar to beta particles.

Nuclear emulsions are available from Eastman Kodak in the United States of America and Kodak, Ltd. and Ilford, Ltd. of England in a variety of forms. American Kodak provides nuclear emulsions in a variety of sensitivities and thicknesses coated on glass plates the size of microscope slides in several thicknesses, with and without a gelatin protective coating. In order of ascending sensitivity these emulsions are designated: NTA, NTB, NTB2 and NTM3. NTB2 and NTB3 are the most commonly used in biological work. These emulsions may be obtained in bulk, also. Kodak also supplies stripping film in several forms. British Kodak supplies several types of emulsion, of which their AR10 stripping film is quite popular in the United States and is now being distributed by the American branch, but it is not available in bulk. Ilford also provides a variety of emulsions as stripping film or in bulk. When not in use, nuclear emulsion should be stored at 5°C. It will keep up to six months or longer if not exposed to too much radiation. The ways in which the various forms of nuclear emulsion are utilized will be discussed below. HERZ [7] and YAGODA [8] have discussed the fundamental interactions of radiation and nuclear emulsions.

RADIOAUTOGRAPHIC PROCEDURES

Dozens of modifications of the few basic methods of radioautography have been evolved, and many of them have been described by BOYD [9]. There are also extensive bibliographies gathered by PASSALAQUA [10] and JOHNSTON [11, 12]. A few of the most generally applicable methods will be described to indicate the possibilities which in practice are limited only by the ingenuity of the investigator.

The two most widely used methods have come to be known as the dipping technique [13, 14] and the stripping technique [15]. Both yield excellent results with characteristic high resolution and dependability. Of the two, the dipping technique, which was developed in this laboratory, is the simpler and more convenient. All techniques have many manipulations in common, and the dipping technique will be described in some detail as a model for the others.

In our laboratory the procedure is as follows. If it is feasible, the mounted tissues should be rehydrated after paraffin or other embedding medium has been removed. This step will aid in obtaining even layers of emulsion which adhere to the tissue and glass much better than if the slides are not rehydrated, and it should be performed unless there is danger of loss of the radioisotope. While the tissue slides are going through the last stages of processing, the light-tight [14] container of bulk nuclear emulsion is taken from the refrigerator and placed in a water bath which is set to bring the emulsion to a temperature of approximately 40°C, in the darkroom. If thinner emulsion layers are desired, the temperature may be raised but not above 50°C (to avoid heat sensitization artifacts). When the emulsion is at temperature and the slides are ready, the slides are taken from the water in which they were rehydrated and arranged in a convenient array where they may be easily grasped at the label end in the dark. If too many slides are removed at once, the last ones to be done will be dried out and may lose some cytological detail. Groups of ten are convenient. The lights are shut off and the series 1 or 2 safelight turned on. Nuclear emulsions are quite insensitive to red light. The cover is then removed from the emulsion container and a clean glass rod is used to stir the emulsion thoroughly but gently without introducing bubbles. Stirring is necessary because during standing of the melted emulsion silver halide settles out of the gelatin. Once the stirring is completed, the first slide is picked up and immersed long axis vertical so that the tissue goes about half an inch below the surface. It is immediately withdrawn and allowed to drain for a few seconds to a minute on to a gauze pad held in the other hand. Within limits, the longer the drainage, the thinner the emulsion layer and the better the resolution. With 10-sec drainage, the dry layer thickness will be about 5 μm. Emulsion must not be allowed to drain back into the container. After draining, the gauze pad is used to remove all emulsion from the bottom of the slide. The slide is then placed horizontally in a tray to gel and be exposed. The slides need be kept horizontal no longer than the ten to twenty minutes it takes the emulsion to gel, after which any position of the slide will be all right. When the tray is full, it is placed in an exposure chamber specially made for the purpose* and other trays used if the number of slides require it. The proper compartments of the chamber are then charged with Drierite and solid CO_2, and the cover is sealed on. The chamber seals itself by means of an oil valve after all the CO_2 has sublimed. As the gas evolves, it is led through the chamber in such a manner that the air is completely swept out. If precautions to eliminate oxygen and reduce moisture below 15% relative humidity are not taken, serious losses of latent image may occur during the exposure period.

There has been much discussion about the proper temperature in the exposure chamber [13], but the best temperature to use is between 18 and 24°C. The argument that less background or fogging occurs at low temperatures has been advanced. In reality, the reduction of fog is due to the reduced sensitivity of the emulsion in the cold and the ratio of useful image

* The complete equipment for the dipping technique, consisting of an exposure chamber and the necessary ancillary equipment, is available from Controls for Radiation, Inc., 130 Alewife Brook Parkway, Cambridge 40, Mass.

to fog probably remains the same. With the dipping technique, fog is very seldom a problem and, therefore, it is preferable to expose at room temperature to obtain a more useful image. The fog level averages three to five grains per 100 μm^2 in this laboratory. However, the dipping technique is perfectly amenable to low-temperature exposure, should an investigator prefer to do so, or if there is an experimental reason for it, the exposure box may be placed in a cold room after it has been sealed.

Exposure time varies according to sensitivity of the emulsion, the total dose of isotope administered, its effective half-life, the length of time between administration and sacrifice of the animal, the degree of concentration, the pattern of distribution in the tissues under study, the thickness of the tissue slices and the energy of the radiation. These factors are difficult to reduce to a formula which could indicate actual exposure times. It has been found simpler to use replicate slides and to develop a few at intervals of several days until optimum exposure times become apparent. One soon gains experience enough to estimate the initial exposure period.

When the initial exposure period has elapsed, photographic development is performed with ordinary glass or plastic staining dishes containing the developing solutions. Ten to twenty slides are placed in the usual staining rack for immersion in the solutions. The solutions used are Kodak D 19 developer, Kodak SB5a stop bath and Kodak Fixer. D 19 is a rapid, fine-grain, fairly stable developer in wide use in radioautography, although others are in use also. The SB5a stop bath is non-swelling, thereby reducing the probability of dimensional changes and silver grain shifting. The acid fixer hardens the emulsion layer as it fixes, which helps prevent damage to, or loss of, the emulsion layer during washing or subsequent handling.

The development time can be as short as one minute or less or prolonged to ten minutes or more. The longer the time, the more granules appear, but increasing the development time too much will frequently increase the background without increasing the useful image proportionately. The photographic processing schedule is as follows:

Kodak D19 developer	1 to 10 min
Kodak SB5a Stop Bath	15 s
Kodak Fixer	2 × clearing time
Wash, running tap water	1 h.

All solutions should be 18 ± 1°C. If the individual solutions vary appreciably in temperature, the likelihood of reticulation increases. Higher temperatures increase the likelihood of the emulsion layer washing off. If it is impossible to achieve 18°C or lower, tropical processing methods can be used. After the slides have been in the fixer for a minute, sufficient white light may be used to observe the time it takes to clear the emulsion, and then the slides should be left in the fixer for an additional period equal to twice the clearing time. Thorough washing in running tap water at 18°C removes all the fixer and insures that no image loss or crystallization of hypo will occur if the preparations are stored for long periods. After thorough washing (or staining) has been completed, cover-glasses can be mounted in the usual way and the preparations can then be studied as soon as the mounting medium is hardened.

The radioautograms prepared this way can be studied under the microscope as though they were ordinary histological preparations. Under low and high dry magnification, only slight adjustment of the fine focus enables one to correlate the histologic with the radioautographic image. Oil immersion requires somewhat greater adjustment of focus, but it is still quite convenient. Similarly, photomicrography is quite simple under the lower magnifications by using a compromised focus, but it is necessary to make two negatives if oil-immersion magnification is required because the focal plane is too thin to focus clearly on both the tissue and the emulsion layers at once. A very simple registration frame suggested by Hoecker and described in BOYD [9] permits very accurate registration of the negatives for printing. Thus, study and recording of the data obtained by radioautography are simple.

BASERGA [16] has very cleverly modified the dipping technique to permit radioautography with doubly-labelled material using tritium and radiocarbon. The modification consists of proceeding essentially as described above but adding a layer of celloidin thick enough to absorb the weak tritium emanation over the developed radioautogram and redipping and developing to obtain a second radioautogram over the first, consisting of the granules exposed only by the radiocarbon.

HAMPTON and QUASTLER [17] use fluid emulsion in conjunction with electron microscopy.

Stripping-film technique differs (as do the other techniques to be described) from dipping primarily in the method of application of the nuclear emulsion to the specimen and also because a film of emulsion is used rather than a fluid emulsion. Specimen preparation and photographic development are similar to the dipping technique. The emulsion is applied by detaching or "stripping" the nuclear emulsion from the temporary glass or acetate support upon which it is placed by the manufacturer and floating it on a water bath. The specimen on the glass slide is then dipped under the floating film and brought up out of the bath with the film adhering to it. When the emulsion has dried, it usually adheres quite tightly to the slide and specimen because it shrinks as it loses water. In this method resolution is excellent, and the radiographic image retains its original relationship to the specimen. The only drawbacks to this technique are the difficulty most people encounter in handling the thin unsupported emulsion: it is quite time-consuming and there is a possibility of radioisotope loss in the water bath.

Stripping film in its dry state has been used by FITZGERALD [18] to apply to dry specimens where it was desirable to avoid loss of water-soluble isotopes. While this technique may create some artifacts due to the rather cavalier handling of the emulsion film which is necessary, no other method will serve when very soluble compounds are involved.

The methods described so far are used for high-resolution work with sections of specimens. For radioautography of whole mounts, for instance of a whole plant or a leaf or of a whole small insect, the simple apposition technique is very useful. In this technique the specimen is positioned on a cut film or a plate film protected from light and exposed, usually under pressure (as in a plant press or other device) to obtain close contact. After exposure, the specimen is separated from the film and the film is developed. The main drawbacks to this method are that it is very difficult to correlate

the radioautographic image with the specimen at the microscopic level once the two are separated, and resolution is not good, but it serves well for gross survey work. Several apposition methods are described in BOYD's book [9].

CONTROLS AND ARTIFACTS

Each slide should be used as its own control by observing the number of developed silver granules in natural or artifactual tissue spaces as well as in areas relatively remote from the tissue. In evaluating the slides this background is subtracted. It is also necessary to use non-radioactive control tissue slides in order to account for silver granules which may be due to the presence of reducing substances such as sulphydryl compounds or even reducing reagents which have not been completely washed out of the tissues. Other sources of background are: accidental exposure to white light, cosmic rays, naturally occurring radioisotopes in the containers, slides and tissues, irradiation of emulsion during immersion of the radioisotope-bearing tissue, radiation from adjacent slides in the exposure chamber, mechanical factors and heating. From time to time blank slides should be included to check the fog level of the emulsion; when it gets too high the emulsion should be discarded.

CONCLUSION

It is hoped that this short review will serve as a general guide to any investigator who wishes to take advantage of radioautography to obtain unique information. The techniques are not difficult if the fundamentals are understood, and the variety of techniques available make it possible to utilize radioautography in many types of research problems. Perhaps it should be emphasized that radioautography, as are radioisotopes in general, is a tool and should not be considered as an end in itself. However, for detecting the cytological positions of very small quantities of radioisotopes, radioautography is a very powerful tool, and it can serve the purposes for which this Symposium was convened admirably.

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DISCUSSION

K.K. NAIR: Is it not true that a mere blackening of the autoradiographs need not be an indication of radioactivity in the sections, but could be an artefact resulting from the precipitation of silver halide in certain niches in the section?

D.L. JOFTES: Yes, certainly. The emulsion is heir to many ills. There are usually radioisotopes in the structures which we use to manipulate the emulsion. Even the glass slides frequently contain some K^{40}, which then interferes, and mechanical manipulations heat it up too.

There are many many sources of error in this sort of thing and it is necessary for that reason to use adequate controls, to be very ruthless in analysis and to eliminate radioautograms which seem to be questionable. We have been doing radioautography now extensively for about eight years, and generally there is no question about really good positive radioautographs. The questions always arise when they are powerfully negative. Non-radioactive control tissues should always be included in the early runs to any new experiment involving radioautography. I have previously published a report on the type of artefact to which you refer. I believe it to be the result of differential drying of the emulsion, which is thicker over these tissue interstices; this differential drying causes mechanical strain in the emulsion and so "exposes" it.

M. HASCOËT: What limits of definition can one hope to obtain by using dry stripping films?

D.L. JOFTES: We have not used this technique in my laboratory but I believe the resolution achievable should be only slightly less than with standard stripping film techniques.

M. HASCOËT: Is it possible to work at the cellular level?

D.L. JOFTES: With low-energy isotopes such as H^3 or C^{14}, probably. It seems to me that one cannot achieve an intimate contact between tissue and a dry film as one can by dipping or standard stripping techniques, and this will adversely affect the resolution.

M. HASCOËT: I have had great difficulties in using a similar method with S^{35}. It is quite difficult to localize sulphur finally at the cellular scale and I would have been happy to know what methods you used to facilitate this.

D. L. JOFTES: That is partly a function of the mean path length of the radiation in the tissue and in the emulsion. I would expect you to get results with S^{35} comparable to those of C^{14}. Unfortunately I myself have no experience with the dry-film technique.

M. FRIED: I wondered if you had any experience of using film slides with fresh frozen sections, and the kind of artefact you get under these conditions.

D. L. JOFTES: We have not done this ourselves but we collaborate with another laboratory which has done work on these lines, and my guess is that the main problem would be associated with the melting, if you will, of the tissue fluid unless care was taken to dry the tissue into position. In the dipping system we put these things immediately into a chamber where humidity is very low and, the sections of tissue and the emulsion being very thin, there was very rapid drying, whereas in the fresh-frozen section there is a fair amount of water; and if you have a soluble isotope there, in compound, when this is warmed you may get some motion or some passage into the emulsion at some distance from the tissue.

The reason that I suggest drying and then rewarming is that you lower the sensitivity of a film considerably by chilling it. With the dipping technique and frozen dry sections one sometimes gets a build-up of emulsion in the interstices which occur there and then in the differential drying of the emulsion film, we often see streaks of silver granules running around the edges of the tissues and in the interstices, but this is such a fine kind of artefact that I am sure you would have no trouble.

III.

LABELLED INSECTIDE STUDIES: TOXICOLOGY AND RESIDUES

RADIOISOTOPES IN THE STUDY OF THE FATE OF INSECTICIDES APPLIED TO ANIMALS AND PLANTS

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Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPES IN THE STUDY OF THE FATE OF INSECTICIDES APPLIED TO ANIMALS AND PLANTS. The use of radioisotopes has been of great value in the development of systemic insecticides for the control of insect pests of both plants and animals. General patterns of absorption, circulation and localization, metabolism, and the site and nature of residues have been determined more rapidly through the use of labelled materials than would have been possible by other available methods of analysis.

Information gained in studies with labelled insecticides has enabled entomologists to learn why some chemicals are relatively more toxic to insects than to mammals and why some are safer for use with certain types of mammal than with others. Results of these studies have led to the development of new methods for controlling insect pests. Hitherto unsuspected metabolic pathways have been elucidated and new methods of analysis have been developed.

Labelled insecticides have been essential in developing basic information on plant systemics. Radioisotopes have made it possible to follow the absorption, translocation and metabolism of systemic insecticides in plants. Also, the chemicals can be followed into insects that feed on treated plants and their metabolism and excretion can then be studied in the insects.

By utilizing labelled materials it has been shown that cotton plants grown from seed treated with systemic insecticides absorb less than 5% of the applied dose. Other studies have demonstrated that systemic insecticides are not readily translocated from treated leaves to new growth.

The use of radioisotopes in studying systemic insecticides will increase in the future. More emphasis is being placed on basic physiological studies on the fate of insecticides in plants, animals, and insects. Another area where isotopes should be used rather extensively is in studies designed to improve methods of application of systemic insecticides.

EMPLOI DES RADIOISOTOPES DANS L'ÉTUDE DE LA TRANSFORMATION DES INSECTICIDES ADMINISTRÉS AUX ANIMAUX ET AUX VÉGÉTAUX. L'emploi de radioisotopes s'est révélé très utile pour la mise au point d'insecticides endothérapiques pour la lutte contre les insectes nuisibles aux végétaux et aux animaux. On a pu déterminer plus rapidement divers processus généraux - absorption, circulation, localisation, métabolisme, emplacement et nature des résidus - grâce à des produits marqués que par les méthodes d'analyse utilisées auparavant.

Les renseignements obtenus à la suite des études faites au moyen d'insecticides marqués ont permis aux entomologistes de découvrir pourquoi certains produits chimiques sont plus toxiques pour les insectes que pour les mammifères et pour quelles raisons l'emploi de quelques-uns d'entre eux présente moins de danger pour certains mammifères que pour d'autres. Les résultats de ces études ont permis de mettre au point de nouvelles méthodes de lutte contre les insectes nuisibles. On a pu aussi expliquer certaines transformations métaboliques insoupçonnées jusqu'à présent et élaborer de nouvelles méthodes d'analyse.

L'emploi d'insecticides marqués a joué un rôle essentiel dans le rassemblement de données fondamentales sur les propriétés endothérapiques des plantes. Les radioisotopes ont permis, en effet, de suivre l'absorption, la translocation et le métabolisme d'insecticides endothérapiques dans les plantes. On peut aussi suivre les produits chimiques dans les insectes qui se nourrissent sur les plantes traitées et étudier ainsi leur métabolisme et leur excrétion.

En utilisant des produits marqués, on a pu montrer que les cotonniers issus de semences traitées au moyen d'insecticides endothérapiques absorbent moins de 5% de la dose appliquée. D'autres études ont prouvé que les insecticides endothérapiques ne sont pas facilement transportés des feuilles traitées aux nouvelles pousses.

L'emploi de radioisotopes dans l'étude des insecticides endothérapiques est appelé à s'étendre, en particulier pour les études physiologiques fondamentales sur la transformation des insecticides dans les plantes, les animaux et les insectes. Les radioisotopes devraient aussi être largement utilisés dans les études visant à améliorer les modes d'application des insecticides endothérapiques.

РОЛЬ РАДИОИЗОТОПОВ В ИЗУЧЕНИИ СУДЬБЫ ИНСЕКТИЦИДОВ ПРИ ОБРАБОТКЕ ИМИ ЖИВОТНЫХ И РАСТЕНИЙ. Использование радиоизотопов оказалось весьма ценным при выработке системных инсектицидов для истребления насекомых-вредителей растений и животных. Общая картина поглощения, циркуляции и локализации, метаболизма, а также местонахождения и характера остаточных количеств инсектицидов были более быстро определены с помощью меченых материалов, чем это было возможно при других методах анализа.

Сведения, приобретенные энтомологами с помощью меченых инсектицидов, дали им возможность выяснить, почему некоторые из химикалий относительно более токсичны для насекомых, чем для млекопитающих, и почему некоторые из них представляются более безопасными для одних видов млекопитающих, чем для других. Результаты этих исследований привели к выработке новых методов истребления вредных насекомых. Были выявлены новые неизвестные до сих пор пути метаболизма и выработаны новые методы анализа.

Меченые инсектициды оказались чрезвычайно важными для получения основной информации о растительных системах. Применение радиоизотопов дало возможность проследить за поглощением, перемещением и метаболизмом системных инсектицидов в растениях. Помимо этого, удалось проследить пути попадания химикалий в организм насекомых, питающихся обработанными химикалиями растениями, а на насекомых могут быть изучены метаболизм этих химикалий и их выделение.

Использование меченых материалов дало возможность показать, что хлопковое растение, выращенное из обработанных системными инсектицидами семян, поглощает менее 5% примененной дозы. Другие исследования показали, что системные инсектициды не легко переносятся из листьев, обработанных инсектицидами, в новые ростки.

Использование радиоизотопов для изучения системных инсектицидов получит в будущем дальнейшее распространение. В настоящее время уделяется больше внимания основным физиологическим исследованиям относительно судьбы инсектицидов в растениях, у животных и насекомых. Другой областью, в которой радиоизотопы должны широко использоваться, являются исследования, направленные на улучшение методов применения системных инсектицидов.

EMPLEO DE LOS RADIOISÓTOPOS PARA ESTUDIAR LOS INSECTICIDAS APLICADOS A PLANTAS Y ANIMALES. Los radioisótopos han sido de gran utilidad en la preparación de insecticidas de acción indirecta para combatir los insectos nocivos para las plantas y los animales. Gracias al empleo de sustancias marcadas se han podido determinar los esquemas generales de absorción, circulación y localización, el metabolismo, el lugar donde se depositan los residuos y el carácter de éstos, con más rapidez que con otros métodos de análisis.

Gracias a los estudios hechos con insecticidas marcados, los entomólogos han podido averiguar por qué ciertos productos son relativamente más tóxicos para los insectos que para los mamíferos, y por qué algunos son menos peligrosos para ciertos tipos de mamíferos que para otros. Los resultados han permitido desarrollar nuevos métodos de lucha contra los insectos. Se han descubierto procesos metabólicos hasta ahora ignorados y se han desarrollado nuevos métodos de análisis.

El empleo de insecticidas marcados ha sido esencial para averiguar datos fundamentales de fitofisiología. Con los radioisótopos se han podido seguir en las plantas los procesos de absorción, translocación y metabolismo de los insecticidas de acción indirecta. También es posible seguirlos en los insectos que se alimenten con las plantas tratadas, y estudiar su metabolismo y excreción.

Por ejemplo, se ha demostrado que las plantas de algodón nacidas de semillas tratadas con insecticidas de acción indirecta absorben menos del 5% de la dosis aplicada. Otros estudios han demostrado que estos insecticidas no pasan fácilmente de las hojas tratadas a los brotes nuevos.

En el futuro se emplearán cada vez más los radioisótopos para estudiar los insecticidas de acción indirecta. Los estudios fisiológicos básicos de los procesos que sufren los insecticidas en las plantas, animales e insectos adquieren cada vez más importancia. También convendría emplear más intensamente los radioisótopos en los estudios destinados a mejorar los métodos de aplicación de los insecticidas de acción indirecta.

INTRODUCTION

Entomologists have used radioisotopes in many areas of research. One of the major fields in which this research has been productive has been that of the fate of insecticides in various biological systems. Through the use of radioactive samples, our understanding of the metabolic fate of insecticides under in vivo conditions has been greatly broadened.

The use of radioisotopes has given entomologists a tool of unparalleled sensitivity for measuring factors such as the rate of penetration or uptake of insecticides by plants, animals and insects. The various and complex reactions, whether intoxications or detoxications, undergone by pesticides in biological tissues have been studied most effectively in efforts in which labelled samples of insecticides have been used. The determination of general patterns of the rates at which insecticides are lost by excretion, or stored in the form of residues, has been greatly aided through the use of labelled materials. Differences in toxicity to different forms, i.e. selective toxicity, have become understood in part through the use of isotopes.

In the present paper, we shall attempt a review of the general principles and patterns of the fate of certain insecticides, as determined by means of these studies.

1. FATE OF INSECTICIDES IN ANIMALS

A. Introduction

Within the necessarily brief confines of the present review, the discussion will be restricted largely to comments on the fate of selected organophosphorus insecticides in warm-blooded animals. The entire area of this subject matter, particularly as it has to do with the development of systemic insecticides, has been reviewed extensively in recent years [8, 39, 40, 41, 55, 57]. Perhaps the most thorough recent review is that of O'BRIEN in his book "Toxic Phosphorus Esters" [38]. For the most part, the present paper will be restricted to general comments on work published before 1960, with somewhat more detailed comments on certain papers published in the past two years.

B. General patterns of the fate of systemic insecticides in animals

Early in their development, organophosphate insecticides were found to be more satisfactory for use on domestic animals than the chlorinated insecticides. The chief advantages of the organophosphates were the lack of persistent insecticide residues stored in the tissues of treated animals and the low levels or absence of insecticide in milk. Early analyses were generally made by means of chemical methods, each method being specific for the compound studied.

Extensive work with radiolabelled samples of organophosphates, for the most part prepared with P^{32}, verified the earlier findings. A long series of papers on the fate of labelled organophosphates in large animals has been

published in the United States in the last seven years. These studies have indicated that, in most instances, the organophosphates are rapidly absorbed by animals after dermal, oral, or intramuscular treatment. Following absorption, small amounts of the insecticides may be found in the blood of treated animals for a few hours. Degradation to non-toxic water-soluble compounds, which are then excreted via the urine, is rapid. The entire process is usually 90% or more complete within 48 h. Small amounts of radioactivity may partition into fat or be excreted in the milk. Generally, only a portion of this radioactivity is in the form of non-degraded insecticide, and even this portion is rapidly dissipated.

Within this overall pattern, considerable variation has occurred in the rates at which the insecticides undergo destructive metabolism. The variations observed have, for the most part, been a reflection of the relative solubility of the compounds studied. The more soluble the compounds are found to be in polar solvents such as water, and the lower their solubility in the more inert and non-polar solvents such as chloroform and hexane, the more rapidly the compounds and residues associated with them are dissipated.

C. Selected examples

Dipterex (dimethyl 2, 2, 2-trichloro-1-hydroxyethyl phosphonate) is an example of one of the organophosphates which undergo extremely rapid degradation in biological systems. The insecticide is soluble to the extent of about 20% in water. Partitioning data have shown that when a sample of the radioactive insecticide is partitioned with equal volumes of chloroform and water, about 45% remains in the aqueous layer [2]. This result is in contrast to what occurs with most of the organophosphates, which can be recovered from aqueous solvents in a near-quantitative manner by a single extraction with chloroform.

Dipterex was the first of the insecticidal organophosphates to be studied in detail following administration to cattle. In 1956, ROBBINS et al. [46] reported on the fate of P^{32}-labelled Dipterex in a lactating cow following administration of a single oral dosage of 25 mg/kg. Radioactivity equivalent to 15 ppm appeared in the blood at 2 h after treatment and then declined to less than 1 ppm at 18 h. However, of the peak level of 15 ppm in the blood, only 7.5% behaved as Dipterex; the other 92.5% represented material degraded within 2 h of administration. There was no evidence that any unmetabolized insecticide was excreted in the milk, although 0.2% of the administered radioactivity was recovered in it. The major means of elimination of the insecticide was via the urine, the peak of excretion occurring 2.5-5.5 h after treatment. Only 3% of the administered radioactivity was recovered in the faeces, an indication that the insecticide was readily absorbed from the gut.

Similar studies on the fate of Dipterex in a dog and in rats have been reported [2, 3]. In these studies, evidence was obtained that degradation in smaller mammals, as in the cow, proceeds rapidly and is virtually complete within two days of treatment.

Co-ral (O, O-diethyl O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) is an insecticidal organophosphate with solubility properties much different from those of Dipterex. Unlike Dipterex, Co-ral is almost

completely insoluble in polar solvents; further, it is not even very soluble in many of the more common non-polar solvents. This difference in solubility properties of Co-ral results in a metabolic picture in animals significantly different from that of Dipterex.

When radioactive samples of Co-ral were sprayed on Hereford bulls, the major portions of the radioactivity were still present on the hide of treated animals 14 d after treatment [46].

Poor absorption of Co-ral was also demonstrated by analysing urine and faeces for radioactivity; less than 10% of the applied dosage was excreted via these routes. With Dipterex, absorbed radioactivity was almost quantitatively excreted; with Co-ral, considerable amounts of residual radioactivity were present in normal phosphorus-containing tissues and organs, evidence that the compound was degraded completely to phosphoric acid. Residues behaving as unmetabolized insecticide were less than 1 ppm in all tissues within two weeks of treatment.

In another study, the fate of radioactive Co-ral in cattle and goats following dermal treatment was investigated [29]. Again large radioactive residues behaving as inorganic phosphate were found in several tissues. Species differences were observed. For example, bone contained high levels of radioactivity in the cow, but low residues in the goat. Poor absorption of Co-ral following oral administration was found in the rat [29, 30]. In both studies, considerable unmetabolized insecticide was excreted in the faeces, evidently never having been absorbed from the intestinal tract.

D. Recent studies

Recently published work has tended to verify the results of earlier studies as to the nature of the metabolism of organophosphates in warm-blooded animals. The occurrence of interspecific metabolic differences has been investigated and the nature of residues present in other domestic animals studied.

In a recent study, the fate of P^{32}-dimethoate (O, O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate) in sheep was determined [11]. As in earlier studies involving cattle [12, 28], the insecticide was rapidly degraded to non-toxic products. In this study, paper chromatographic evidence was obtained which indicated that cleavage of the carbonyl-nitrogen bond was the main site of metabolic detoxication and confirmed previous findings of studies in which ion-exchange chromatographic procedures had been utilized. Residues behaving as unmetabolized insecticide were practically nil in tissues of animals sacrificed 2 and 4 weeks after treatment.

There are no published papers on the fate of P^{32}-labelled Ruelene (O-4-tert-butyl-2-chlorophenyl O-methyl methyl-phosphoramidate), an insecticide with systemic properties, in large animals. However, in poultry, it was determined that the insecticide was rapidly degraded after oral administration [10]. No evidence was obtained for the presence of insecticide in the eggs of treated hens. Similar work in poultry with P^{32}-labelled Co-ral [16] has indicated that small insecticide residues may be present in eggs after dust treatment. As in work with cattle, these differences are probably a reflection of differences in solubility properties and ease of metabolic detoxication of the two insecticides.

Another material of solubility properties similar to Co-ral is Bayer 22408 (O, O-diethyl O-naphthalimido phosphorothioate). In cattle, following oral treatment with radioactive samples of the insecticide, GATTERDAM et al. [22] found that more P^{32} was eliminated via the faeces than via the urine. Significant portions of the radioactivity present in the faeces occurred as undegraded insecticide and its oxygen analogue. The authors suggested that the insecticide might be effective against coprophagous insects, although it had proved to be ineffective as a systemic insecticide against several species of livestock pests.

Following dermal administration of the same insecticide to Holstein cows, BUTTRAM et al. [9] determined that residues of less than 0.1 ppm were present in the milk. Curiously, from 32 to 40% of the applied dosage was excreted in the faeces, with as much as 22% occurring as non-degraded insecticide. The residues of insecticide in the faeces were toxic to stable-fly larvae introduced into samples collected 2-7 d after treatment. The toxicity data obtained agreed well with those of radioactive analyses.

E. Future uses of radioisotopes in animal systemic research

The use of labelled samples of insecticides has proved an excellent tool in systemic insecticide research. The methods of analysis developed in these studies and the general pattern of results elucidated have been applicable to other studies involving the fate of labelled insecticides in insects and mammals. Future utilization of labelled insecticides will probably be made in studies involving isotopes other than P^{32}. Thus, the World Health Organization is currently making samples of C^{14}-labelled malathion available to interested entomologists, and a study on the fate of a tritiated sample of Famophos (O-p-(dimethylsulfamoyl) phenyl O, O-dimethyl phosphorothionate) in mammals and insects has been reported [49].

A new usage of organophosphate insecticides, derived as a direct result of the radioactive studies, was developed for the control of faeces-breeding flies; such control is obtained by feeding the insecticides to the animals so that their faecal deposits will be toxic to insects breeding therein. Thus, both Co-ral and Bayer 22408, materials of such poor solubility that they are never absorbed from the digestive tract, have proved effective in controlling faeces-breeding flies [1, 19]. Similar studies on the control of flies breeding in poultry droppings have been reported [17, 50].

That the use of labelled compounds is not the sine qua non of metabolic studies has been shown recently in work by HOPKINS et al. [26]. These workers have demonstrated through the use of enzymatic rather than radioassay techniques that active antiesterase materials other than the oxygen analogues of phosphorothioates may be produced and be responsible for toxic effects. Working with ronnel (O, O-dimethyl O-(2, 4, 5-trichlorophenyl) phosphorothioate), they found the parent insecticide and its oxygen analogue present in the blood of treated cattle; in addition cattle grubs from the same animal contained ronnel and an unidentified anticholinesterase other than the oxygen analogue. The authors did not speculate on the nature of the unknown material.

As in plant systemic work, no problems of technique have arisen in the use of labelled systemic insecticides. Not all radioactivity present in tissues

is insecticide; indeed, not even all radioactivity soluble in organic solvents is insecticide. These problems of analysis have been solved in part through the use of a hexane-acetonitrile clean-up partitioning procedure. However, even this tool has proved to be less than perfect, since it has been shown that feeding labelled phosphoric acid to animals results in the occurrence of acetonitrile-soluble radioactive residues [18]. Thus, in order to prove the presence of toxic residues the employment of paper and column chromatographic techniques of analysis as well as enzymatic assays has been necessary. The extreme sensitivity of these alternative methods can, in certain instances, negate the advantages of sensitivity obtained through the use of labelled samples of insecticide.

2. FATE OF INSECTICIDES APPLIED TO PLANTS

A. Introduction

The portion of this report dealing with the fate of insecticides in plants will be limited to a general review of organophosphorus systemic insecticides. Systemic insecticides, as used to combat phytophagous insect pests, are compounds which are absorbed by a plant and translocated to other parts of the plant in large enough amounts to be toxic to insects feeding thereon. The first practical demonstration of this method of insect control was reported by HURD-KARRER et al. [27], who found that selenium was absorbed by the roots of wheat plants and translocated to the aerial plant parts in sufficient quantities to control aphids. However, little interest was shown in this area of research until SCHRADER [48] discovered the systemic activity of certain organic insecticides. Since this time, considerable work has been done on plant systemic insecticides.

Nearly all of the organic insecticides used at the present time possess some degree of systemic action. Most of the insecticides which are commonly called non-systemic will penetrate into the leaf, stem or roots of plants. This property has been called local systemic or Tiefenwirkung action.

METCALF [34] has stated that systemic action in plants is controlled by the following characteristics of an insecticide: (1) ability of the compound to penetrate into the plant; (2) sufficient water-solubility to move with the transpiration stream; and (3) sufficient stability in the plant so that the insecticide or its toxic metabolic products can exert the desired insecticidal action. These criteria are related to the physical and chemical properties of the insecticide as interrelated with the physiological action of the plant.

Several excellent reviews have been published recently on systemic insecticides [4, 37, 44]. Recent books by O'BRIEN [38] and HEATH [25] contain much information on systemic insecticides. In the following discussion some of the major findings in the areas of absorption, translocation and metabolism of systemic insecticides by plants will be briefly reviewed. A large portion of the data pertaining to these studies was obtained with the use of radiolabelled compounds. Without radioisotopes, much of these data would not be available because precise quantitative and qualitative chemical analytical methods are also not available. More information is available on

metabolism of systemic insecticides than on absorption and translocation, principally because of the emphasis recently placed on insecticide residues on and in food and fibre. Nearly all countries require residue data of some kind before an insecticide can be marketed.

B. Absorption

As previously mentioned, the absorption of organic insecticides by plant tissue is not uncommon. This absorption is undoubtedly due to the lipoid solubility of these compounds. In practice, systemic insecticides are usually applied so that they are absorbed by either the roots or leaves. Some experimental work has been done on stem treatment.

Using P^{32}-labelled compounds, DAVID [13, 14] demonstrated that Schradan (octamethyl pyrophosphoramide) was selectively rejected by bean roots but Dimefox (tetramethylphosphorodiamidic fluoride) was selectively absorbed. METCALF et al. [35] reported that P^{32}-labelled Schradan was absorbed by lemon roots at the same rate as $H_3P^{32}O_4$ from water cultures. TIETZ in a comprehensive report of studies with P^{32}-labelled demeton (Systox) (O, O-diethyl S(and O)-2-(ethylthio)ethyl phosphorothioate) [54], concluded that the toxicant was absorbed readily by the intact root. The most rapid absorption was observed in plants growing in water culture; slower absorption took place in sand and soil cultures. Using beans grown in water culture, this worker found that the roots selectively absorbed the insecticide during the first day but thereafter absorbed water and the insecticide at about the same rate.

Recent reports by HACSKAYLO et al. [23, 24] with P^{32}-labelled phorate (O, O-diethyl S-(ethylthio) methyl phosphorodithioate) and P^{32}-labelled dimethoate (O, O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate) discussed the absorption of these insecticides by the roots of cotton plants. The absorption of P^{32}-labelled phorate from nutrient solutions by cotton roots was very rapid initially but decreased with time. A leakage of the absorbed phorate from the roots back into the nutrient solution was observed. This leakage indicated that the insecticide caused some damage to the root system of the treated plants. In studies with P^{32}-labelled dimethoate, it was found that this compound was selectively rejected by the roots of cotton plants. The roots of cotton plants grown in complete and phosphorus-deficient water cultures absorbed essentially the same quantities of dimethoate.

LINDQUIST et al. [32] exposed small sections of the roots of intact cotton seedlings to P^{32}-labelled phorate and $H_3P^{32}O_4$. They found that the amount of phorate absorbed was in direct proportion to the volume of the root exposed to the insecticide. No evidence was found to indicate that any portion of the root absorbed phorate preferentially. However, $H_3P^{32}O_4$ was actively absorbed by the cotton root from 20 to 40 mm above the root tip. The volume of the root exposed to $H_3P^{32}O_4$ did not influence the amount absorbed. These workers concluded that the mechanism of absorption of phorate and that of $H_3P^{32}O_4$ by cotton roots were not similar.

Seed treatment with systemic insecticide for the control of insects attacking young plants has received considerable attention during the past few years. This method of applying systemic insecticides has been recently reviewed [42]. DAVID et al. [15] studied the absorption of P^{32}-labelled

demeton thiol isomer by seeds. These workers concluded that the demeton thiol isomer was absorbed directly into the cotyledons as the broad-bean seeds germinated and that some of the toxicant was absorbed by the roots of the seedling after emergence. REYNOLDS et al. [43] reported on a series of experiments with cotton, alfalfa and sugar-beet seed treated with P^{32}-labelled phorate, demeton thiol isomer and Di-syston (O, O-diethyl S-[2-(ethylthio) ethyl] phosphorodithioate). These workers demonstrated that the amount of toxicant absorbed by the plants following seed treatment was about the same as that following application of granules in the seed row. From 3 to 9% of the dosage applied was recovered in the resulting plants 16 d after planting. Similar studies with P^{32}-labelled phorate [33] demonstrated that absorption of this insecticide following seed treatment takes place primarily during the first few days after planting. In standard laboratory germination tests, phorate-treated intact cotton seeds absorbed 0.7% of the applied dose in 5 d. However, with the seed coats removed before treatment, the seeds absorbed 11.9% of the applied dose. Furthermore, more dehulled seeds germinated in the test, and germinated faster, than intact seeds. For comparison, intact and dehulled seeds were treated with $H_3P^{32}O_4$ and the radioactivity absorbed by the germinating seeds was measured. The presence of the cotton seed hull did not reduce the absorption of the $H_3P^{32}O_4$ as it did the absorption of phorate. Dimethoate is absorbed by cotton plants grown from dimethoate-treated seeds to about the same extent as phorate [31]. From 4 to 6% of the applied dose of phorate or dimethoate was found in the cotton plant. Bidrin (Shell 3562; 2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) is absorbed by cotton seeds to approximately the same extent (LINDQUIST et al., unpublished data). In conclusion it can be stated that with the aid of radio-isotopes it has been found that the seed-treatment method is not a very efficient way to get a systemic insecticide into a plant.

MITCHELL et al. [37] discussed the absorption of systemic insecticides following stem or bark application. In general, this method of applying the toxicants is as effective or more effective than root treatment. Preliminary results with P^{32}-labelled Bidrin indicate that the toxicant is very rapidly absorbed by cotton stems (LINDQUIST et al., unpublished data). Data presented by BOWMAN et al. [6] indicated that phorate and Chipman R-6200 (O, O-diethyl S-(2-diethylaminoethyl) phosphorothioate p-toluenesulfonate) were readily absorbed by cacao trees when the materials were implanted into the tree trunks. SANTI [47] demonstrated that dimethoate (Rogor) with tri-n-butylphosphate added was rapidly absorbed by lemon-tree trunks.

Foliar sprays of systemic insecticides probably are more widely used than any other method of application. However, most of the insecticidal activity of these insecticides is local systemic action, i.e., penetration into the leaf. It is apparent, therefore, that absorption of systemic insecticides by leaves is quite important. MITCHELL et al. [37] compiled data from several sources relative to the rate of penetration of systemic insecticides into lemon leaves. Dimefox was absorbed more rapidly than Schradan, demeton, or amiton (O, O-diethyl S-2(diethylaminoethyl) phosphorothioate hydrogen oxalate). Several studies have been conducted on the absorption of P^{32}-labelled systemic insecticides by leaves [5, 13, 14, 52, 53, 54]. In general, these authors found that the compounds were not absorbed through the stomata and that light and temperature influenced the rate of absorption.

Although methyl parathion (O, O-dimethyl O-p-nitrophenyl phosphorothioate) is not regarded as a systemic insecticide, recent studies [51] have shown that this compound is readily absorbed by cotton leaves. By using P^{32}-labelled material, maximum absorption was found to take place within 2 h, after which the concentration within the leaf gradually decreased. Studies on the penetration of P^{32}-labelled Bidrin into excised cotton leaves indicated that maximum absorption was effected after 4 h when the leaves were held in the light, but took 24 h when the leaves were held in the dark (LINDQUIST et al., unpublished data). No difference in the rate or amount of insecticide absorbed was found when leaves were treated on the upper or lower surfaces.

C. Translocation

The translocation of systemic insecticides from the roots to the aerial plant parts appears to be via the transpiration stream in the xylem. Recent reports by HACSKAYLO et al. [23, 24] supported this observation. These authors also demonstrated that, following the initial rapid absorption of phorate by cotton roots, the insecticide was rapidly translocated upward into the leaves, thus reducing the phorate concentration in the roots. Factors which governed transpiration, i.e., temperature and humidity, also governed the amount of phorate accumulated in the leaves. LINDQUIST et al. (unpublished data) have shown that the translocation of dimethoate to cotton leaves, after application of the toxicant to the soil, follows the transpiration stream. Experiments were conducted in which half of the plant was held under conditions which slowed transpiration and the other half under conditions which were favourable to transpiration. The results of these tests demonstrated that dimethoate accumulated in much larger quantities in leaves held under favourable transpiration conditions.

Translocation of systemic insecticides following seed treatment appears to be similar to translocation following root treatment [37]. However, as previously stated, considerable amounts of the insecticide are absorbed directly by the germinating embryo. In fact, LINDQUIST et al. [33] have shown that phorate-treated cotton seeds absorb most of the toxicant that will eventually be absorbed before the young seedlings emerge. This result indicated that there is little actual translocation of the toxicant via the root system following seed treatment.

The translocation of systemic insecticides following stem treatment has not been investigated very thoroughly. WEEDING [56] demonstrated that S^{35}-labelled demeton was translocated upward and downward in lemon seedlings after stem application. Most of the movement was upward. Translocation took place initially in the xylem but gradually diffused into the phloem. LINDQUIST et al. (unpublished data) found that P^{32}-labelled Bidrin was translocated almost entirely upward when applied to the stems of cotton plants. METCALF et al. [36] found that P^{32}-labelled demeton thiono isomer was translocated to the leaves of lemon seedlings after stem treatment in a pattern similar to that noted after root treatment.

More research has been reported on the translocation of systemic insecticides after foliar application than on translocation after other methods

of treatment. In general, these insecticides are not translocated in large enough quantities to be insecticidally active. However, if the entire plant is treated, sufficient translocation takes place to the new growth to give insect protection. Upward translocation takes place much more readily than downward translocation. THOMAS et al. [52] reported that both temperature and light are important in translocation. Light appeared to be the most important factor. The translocation of these toxicants following foliar application probably is related to photosynthate movement in the plant [37]

D. Metabolism

Since the metabolism of systemic insecticides has been thoroughly reviewed [20, 21, 25, 38], the discussion of this subject here will be rather brief. Almost all of the metabolism studies have been carried out with radiolabelled insecticides. This research has revealed the relatively complex metabolic degradation pathways of many of the systemic insecticides. In a recent report on dimethoate [7], 11 metabolites were detected in various insect and plant extracts. Without the use of radiolabelled compounds research of this nature would not be possible.

One of the major difficulties in measuring the absorption and translocation of systemic insecticides is the rapid metabolism of many of these compounds by the plant [37]. Care must be taken before assuming that all radioactivity found in a plant after treatment with a labelled systemic insecticide is associated with the parent molecule. BULL et al. [7] found that cotton seedlings degraded more than 60% of the absorbed dimethoate in 5 d following root treatment in solution culture. Insects feeding on the treated plants further degraded the toxicants. Additional work along this line is needed to establish the relationship between plant and insect metabolism.

E Future uses of radioisotopes in plant systemic research

Radioisotopes have been used quite extensively in the relatively few investigations concerned with systemic insecticides. In the areas of absorption and translocation, very few critical studies have been done in which the metabolism of a compound has been taken into consideration. This effect of the plant on the insecticide must be known before any real knowledge of the systemic behaviour can be studied. Metabolism, however, is a major study in itself.

It is the writers' belief that the use of radiolabelled systemic insecticides would greatly aid the development of methods of applying these chemicals. Very little work has been done in this area. Nothing is known about the effect of various surfactants on the systemic action of these compounds. Very little has been done to elucidate the movement of systemic insecticides in the soil after granular application. Stem treatment is a very promising method of application. All of these topics can be investigated with much greater precision with radiolabelled compounds than without. In all studies discussed above, however, metabolism of the insecticide must be taken into account.

Other studies which can best be done with the aid of radiolabelled compounds include a determination of the mode of action of the absorption of

systemic insecticides by plants and the physical and chemical processes involved in translocation. Considerably more information must be available in this area before we can understand much about systemic insecticide behaviour in plants.

3. SUMMARY

It should be obvious from the preceding report that radioisotopes have been used quite extensively in studies associated with the fate of insecticides applied to animals and plants. Although residues as such have not been discussed at length, all of the topics covered relate directly to residues. In fact, much of the information cited was obtained during residue studies.

The use of radioisotopes has made it possible to study precisely the absorption, translocation, metabolism and excretion of insecticides by animals and plants. As more data are accumulated on the topics discussed in this paper, it will be possible for chemists to synthesize insecticides for use against specific insect pests. Also, insecticides which are extremely toxic to insects but virtually non-toxic to mammals should be the result of this type of research.

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DISCUSSION

J.W. MILES: I would like to thank the authors for their acknowledgement of the support given by the World Health Organization (WHO) to the work done with C^{14}-labelled malathion. I would also like to mention that WHO has supplies of C^{14}-labelled DDT, dieldrin and malathion for distribution to laboratories for projects involving insects of public health importance. In addition a new carbamate insecticide, O-isopropoxyphenyl-N-methyl carbamate, labelled in the isopropyl group, will soon be available for distribution. Further information can be obtained by writing to the Vector Control Section, Division of Environmental Health, WHO.

W. KLOFT: It is stated in the paper that any systemic insecticide labelled with P^{32} is transported first upwards into the xylem of a plant, and then transported into the phloem sap. Could you please indicate the methods that could be used to prove that?

W. E. ROBBINS (on behalf of F. W. Plapp and D. A. Lindquist): This is dealt with very fully in the work by Mitchell et al cited as reference [37] to the paper.

G. F. BURNETT (Chairman): What is the chance of charging a cow with sufficient radioactivity to mark tsetse flies (Glossina spp.) feeding on it when driven through the bush? These flies feed on nothing but blood and are very difficult to raise in the laboratory There is no convenient way of marking them without capture.

W. E. ROBBINS: It would probably be possible. There are quite a number of studies on the fate of radioisotopes in large animals, and certain of these studies might aid you in selecting the appropriate isotope for high blood levels in the animal and a long biological half-life. About 1952, for instance, Dahm did similar work on a goat. He was comparing parathion and inorganic phosphorus. His work might be of assistance as regards the maintenance of blood levels.

F. T. PHILLIPS: I would like to comment on the use of excised cotton leaves in penetration studies of P^{32}-labelled Bidrin. I have been told that the metabolic processes of excised leaves are different from those of leaves attached to the plant, so perhaps the figures obtained for the excised leaves would not be the same as for leaves which were attached to the plant? Photosynthesis and production of proteins in the plant are not going on, for instance, and that may affect your figures.

W. E. ROBBINS: I think Dr. Casida would like to comment.

J. E. CASIDA: Yes, Dr. Phillips is quite correct, but the intact plant can be treated by injection through the stem to minimize this difficulty. We take a very fine pulled-out glass needle, which is easily inserted into the stem with no apparent damage to the plant. Between 5 and 50 μl of solution put in are taken up by the plant within half an hour to two hours. Then we continue with the study.

SOME PROBLEMS IN THE DETERMINATION OF RESIDUES IN PLANTS AND MAMMALS

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Abstract — Résumé — Аннотация — Resumen

SOME PROBLEMS IN THE DETERMINATION OF RESIDUES IN PLANTS AND MAMMALS. In most instances residues must be determined by chemical or biochemical methods without the use of radioisotopes. These methods assume that the nature of the toxic compounds present is known, and that they can be extracted in known yields. Neither assumption is easily validated except by using radioisotopes. The use of radioisotopes to investigate these problems is described, with examples taken from work on demeton, dieldrin and the fungicide triphenyltin acetate.

PROBLÈMES RELATIFS A LA DÉTERMINATION DES RÉSIDUS DANS LES VÉGÉTAUX ET CHEX LES MAMMIFÈRES. Dans la plupart des cas, les résidus doivent être déterminés par des méthodes chimiques ou biochimiques, sans recourir aux radioisotopes. Ces méthodes sont fondées sur l'hypothèse que l'on connaît la nature des composés toxiques présents et que l'on peut les extraire en quantités connues. Aucune de ces deux conditions n'est facile à réaliser si ce n'est à l'aide de radioisotopes. L'auteur décrit l'utilisation des radioisotopes dans l'étude de ces problèmes et donne des exemples tirés de travaux sur le déméton, la dieldrine et un fongicide (l'acétate de triphényltine).

НЕКОТОРЫЕ ПРОБЛЕМЫ ОПРЕДЕЛЕНИЯ ОСТАТОЧНЫХ ИНСЕКТИЦИДОВ В РАСТЕНИЯХ И У МЛЕКОПИТАЮЩИХ. В большинстве случаев остаточные инсектициды должны определяться химическими и биохимическими способами без применения радиоизотопов. При применении этих методов предполагается, что известен характер присутствующих токсических смесей и что они могут быть экстрагированы в известных количествах. Ни одно предположение не может быть свободно принято, если оно не подтверждено радиоизотопной методикой. Описывается применение радиоизотопов для исследования этих проблем и приводятся примеры, взятые из работ, связанных с деметоном, дильдрином и фунгицидным трифенилацетатом олова.

DETERMINACIÓN DE RESIDUOS DE INSECTICIDAS EN PLANTAS Y MAMÍFEROS. En la mayor parte de los casos la determinación de los residuos de insecticidas debe efectuarse por métodos químicos o bioquímicos, sin emplear radioisótopos. Para estos métodos se parte de dos supuestos: que se conoce la naturaleza de los compuestos tóxicos presentes y que esos pueden extraerse con rendimientos conocidos. Ninguno de estos supuestos puede justificarse fácilmente si no es mediante el empleo de radioisótopos. El autor de la memoria examina este empleo de los radioisótopos y cita ejemplos tomados de trabajos efectuados con los insecticidas Demetón y Dieldrín, y con el fungicida acetato de trifenilestaño.

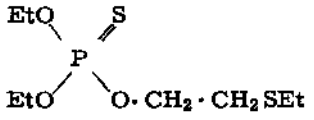
Much of the work in assessing the safety of a pesticide depends upon analysis of residues in crops and animal tissues. A high degree of reliability in methods is obviously essential – residue analysis is not a field in which speculation can be allowed much part – and this sets problems. Some of these problems, and the use of labelled compounds in solving them, are considered in this paper, with examples from work with which the author has been concerned. Not all of this work is new, but little of it has been described in this context.

A problem of very general interest is that of the reliability of recovery tests as usually carried out. In the instance to be described, an apparently reliable method gave substantially nil residues when used on a treated crop, a result which is very acceptable, but also, naturally, suspect. The example is that of Dimefox in cocoa beans.

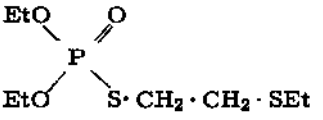
Dimefox is the organophosphorus systemic insecticide N, N'-tetramethylphosphorodiamidic fluoride, $(Me_2N)_2PO \cdot F$. It was proposed at one time for the control of mealy-bugs on cocoa trees (Cacao theobroma) [1]. In view of its high toxicity, only levels below 0.1 mg/kg would be acceptable in the beans at harvest. An adequate analytical method, sensitive to 0.01 mg/kg, was developed. The beans were macerated with an oil of high boiling point. The oil was distilled under reduced pressure, and the relatively volatile Dimefox was carried over quantitatively with the first few per cent of the oil, from which it could be extracted, and estimated as phosphate [2]. This method was applied to beans from treated trees and gave residues of 0.05 mg/kg or less, even when the trees as a whole contained enough Dimefox to kill the mealy-bugs feeding on their foliage. It seemed possible that the recovery tests were at fault: Dimefox might be recovered quantitatively when added to beans just before maceration, but might not be recovered when it reached the beans by a systemic route, when it might either become irreversibly adsorbed or enclosed in plant matrices not destroyed by the distillation process. Pods were therefore left for a few days with their stems dipped in a dilute aqueous solution of Dimefox labelled with P^{32}. The pods were then opened, and the beans were divided into seven samples and analysed for Dimefox. Four samples were analysed in the usual way, except that the phosphate was estimated as P^{32} in a liquid counting tube, and were found to contain 0.044 ± 0.002 mg/kg (means ± SD). The remaining samples were macerated with water or refluxed with chloroform, and the macerates were counted. The extracts were then bulked, and the Dimefox was separated by partitioning between water and chloroform and estimated by counting. The Dimefox content of the beans as found by this completely different method was 0.046 mg/kg, in excellent agreement with the results of the distillation method. The distillation method was thus fully vindicated. These experiments also provided an explanation for the very low quantities found. The macerates in water contained P^{32} equivalent to 4 mg/kg of Dimefox, but 99% of this was as ionic compounds of very low toxicity, i.e. fruit tissue metabolized Dimefox very much more rapidly than leaf tissue did. This conclusion was later confirmed by analysing pods from a tree treated with P^{32} Dimefox. In these experiments the residual Dimefox concentration was too low to measure, but ionic decomposition products were found.

In this type of study the advantage conferred by using the labelled compound is that it can be estimated reliably after a method of extraction which does not separate it from naturally occurring compounds. The necessity for this separation is, of course, the controlling factor in most procedures. When it does not exist, as with labelled compounds, very much more drastic procedures can often be used, and a reliable check to a normal, non-isotopic method can be provided. It would be desirable always to have such checks; and where organophosphorus compounds are concerned this could easily be done almost always.

Another type of problem may arise when there is an obvious discrepancy between the behaviour of an insecticide and the methods suggested. This was the case when Demeton was first marketed under the names Systox and Bayer 8169. Analysis showed that Demeton consisted of two isomers: diethyl 2-ethylthioethyl phosphorothionate, I and O, O-diethyl S-2-ethylthioethyl phosphorothiolate, II [3].

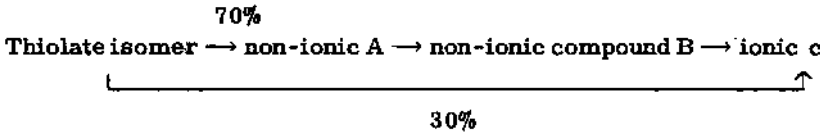


I



II

Tests on toxicity to insects and mammals indicated that the thiolate, II, was about ten times more active than the thionate, I. Both compounds could be extracted from vegetable material by hydrocarbon solvents, and the methods of analysis proposed depended on this type of extraction, followed by biological estimation using insects. Such methods gave very low residues within a few days of spraying, although insects feeding on treated plants continued to die for several weeks. In this case the results could not be explained in the same way as the low residues of Dimefox in cocoa beans, as the insects killed by Demeton were feeding on the actual foliage analysed. Some sugar beet (Beta sativa) and other plants were therefore treated with the thiolate isomer, II, labelled with P^{32}. Foliage was taken at various times after treatment and macerated with water. The macerate was then extracted successively with solvents of increasing polarity, and these extracts were in turn subjected to a simplified form of counter-current extraction. Four discoveries were made. Firstly, the thiolate itself rapidly disappeared, as already indicated by other methods. Secondly, two non-ionic compounds were produced. Thirdly, these apparently fitted a metabolic scheme which can be represented as follows:-



The non-ionic metabolites persisted for a few weeks, so that, if they were insecticidal, their formation could account for the prolonged systemic action of Demeton. Fourthly, neither of the two non-ionic metabolites could be extracted by most hydrocarbon solvents, and would not, therefore, be detected by the methods of analysis suggested. The metabolites were extracted in sufficient quantity for their toxicity to be tested, and as they were nearly as toxic as the parent compound both to insects and mammals it was clear that any worth-while method of residue analysis must record them. This work, which was reported briefly by HARTLEY [4], did not show what the metabolites were, nor did it supply a method of residue analysis; these problems were solved later [5, 6, 7, 8]. Nonetheless, the basis of much further work on those phosphorus insecticides which metabolize to active

compounds in plants was laid down in this preliminary study, which occupied only about 10 weeks. It is now common practice to use tracers in order to establish the presence or absence of toxic metabolites in plants, and their use for this purpose constitutes an essential step in the justification of analytical methods for most compounds with systemic properties.

Methods of analysis of animal tissues and products, meat and milk, do not differ in principle from the analysis of crops, and the value of using labelled compounds is the same. New problems appear, however, in the toxicological studies which are an essential part of the work involved in clearing a pesticide for use.

The initial stage in such investigations consists in determining the acute and chronic toxic effects of the compound in question, and of observations on signs and symptoms of poisoning. After this, some knowledge of the behaviour of the compound in mammals is desirable, with the eventual aim of explaining its mechanism of action. This degree of success is very rare. It has been substantially achieved with organophosphorus pesticides, and is of practical value: recommendations on permissible levels, handling and so on can be made with much more confidence about these compounds than about others whose mechanism of action is largely unknown, such as the chlorinated hydrocarbons. It is clear, however, when the studies on phosphorus anticholinesterases are considered as a whole [9, 10, 11] that tracer studies formed only a small part of the work of establishing the mechanism of action: the contribution of biochemists, pharmacologists and physiologists who did not need to use tracers was far greater. It is in the first stages of an investigation that tracers overcome difficult problems: before one asks "How does this act?", and when the questions are: "Where does this lodge in the body, and for how long; how rapidly is it excreted; and is it metabolized at all?" These questions can be answered only by analyses, and they raise acute problems, because the toxicologist wants to start work with small animals in order to correlate easily measurable effects such as the LD50 values with concentrations in vivo, and the sample sizes are then usually so small that most chemical methods are not sensitive enough. Some work still in progress on the fungicide triphenyltin illustrates these points.

Triphenyltin salts have the structure $(C_6H_5)_3SnX$, where X is an acid group. The acetate, under the name Brestan, has been used to control blight (Phytophthora infestans) on potatoes, and other fungus diseases on celery and sugar beet. The compound is very toxic by intraperitoneal injection, with LD50 values of 10 mg/kg in the rat and 4 mg/kg in the guinea-pig. Oral LD50 values are higher – 450 mg/kg for the rat and 30 mg/kg for the guinea-pig when the compound is given in arachis oil [12] – but the LD50 value in the rat is probably only about 70 mg/kg when the compound is given in dimethylformamide. The animals die 2-7 d after a lethal dose, with no specific symptoms and no obvious pathological lesions. Two questions arose. Firstly, does the compound persist in the body? Delayed death is not good evidence for persistence. Secondly, is the rat less sensitive than the guinea-pig to oral dosing because it absorbs the compound less readily, or because it metabolizes it rapidly before absorption can take place?

A first attempt was made to answer these questions by means of chemical methods of analysis, which appeared sensitive enough [13, 14]. When, however, triphenyltin acetate was left in tissues for 30 min before extraction

was attempted, only one method of extraction was found useful, namely maceration with a mixture of chloroform, methanol and conc. hydrochloric acid (200/100/2 by volume) similar to that used for the extraction of proteolipids [15, 16]. This had to be followed by tedious processing, and then only worked reliably on rat brain.

A specimen of triphenyltin chloride labelled with Sn^{113} (500μc/g) was then obtained from the Radiochemical Centre, Amersham, England. Experiments were carried out on rats and guinea-pigs, but only those on the rat are reported here. Essentially, albino male rats (Porton Wistar strain) were dosed with triphenyltin chloride intraperitoneally in arachis oil or orally in arachis oil or dimethylformamide. Animals were killed at intervals after injection, and specimens were taken for analysis. Urine and faeces were also collected from animals given the compounds orally. The total Sn^{113} content was estimated by scintillation counting in a 5-cm well-type crystal. The efficiency was 27% for a 1-ml specimen and 18% for a 5-ml specimen. Specimens had to be left about 12 h between extraction and counting, to allow equilibration between Sn^{113} and its indium daughter-isotope, In^{113m}, with a half-life of 104 min. Most of the recorded radiation is due to In^{113m}, and the equilibrium is disturbed by many extraction procedures.

The results of distribution studies on rats given triphenyltin chloride intraperitoneally are shown in Table I. The Sn^{113} was distributed throughout several tissues, including the brain, the concentration fell only slowly, and the distribution did not change much with time. In brain tissue measurable concentrations could be found 38 d after treatment. The high concentrations found 2 h after injection in specimens taken from the abdominal cavity probably represent material on the surface from the injection and should be discounted.

TABLE I

CONCENTRATIONS OF TIN IN THE TISSUES OF RATS TREATED WITH TRIPHENYLTIN CHLORIDE INTRAPERITONEALLY

Concentrations are expressed as μg triphenyltin chloride per g tissue. Columns headed 'A' refer to rats given 8.8 mg/kg, columns headed 'B' to rats given 3.5 mg/kg, and column C to rats given 4.4 mg/kg

Tissue	Times after injection						
	2 h	1 d		2 d	4 d		10 d
	A	A	B	A	A	B*	C*
Brain	3.2	5.8	2.6	3.6	4.4	1.2	0.7
Liver	41	28	8.7	10	13	2.2	1.1
Kidney	15	20	8.0	19	14	6.8	4.4
Fat	16	10	3.0	21	-	1.2	0.7
Muscle	2.2	2.7	1.2	2.1	-	0.6	0.4
Heart	8.7	2.2	2.2	3.3	-	-	-
Blood	2.3	1.1	0.5	0.8	-	0.14	0.05

* Average of 3 rats

The results did not show that the compound recorded was triphenyltin. Two methods have been used to investigate this, isotope dilution analysis and the separation procedure described by BOCK et al. [17]. Both agreed in showing that, of the Sn^{113} in rats 3 d after peritoneal injection of 6 mg/kg, 85% in the brain and 25% in the liver and kidney were present as triphenyltin. Bock's method also indicated that 25% in all those tissues was present as triphenyltin 18 d after injection. The method may not achieve complete separation in the presence of animal tissues, and this result is being checked by the isotope dilution method. If it is correct, triphenyltin persists considerably longer in the rat than it was found by HEROK and GÖTTE [18] to persist in the sheep; their evidence for the rapid excretion of triphenyltin was not, however, entirely convincing.

Excretion studies showed that Sn^{113} was retained after oral injection (Table II). The rate of excretion seemed to depend on the dose and medium, but more work is required to establish this. Most of the Sn^{113} excreted was apparently triphenyltin until half of the tin had been excreted. Thereafter some tin was probably excreted as metabolites.

Rats treated orally were also analysed for triphenyltin, with the results shown in Table III. The tin concentrations in most tissues were very similar to those found after equitoxic doses given intraperitoneally, except that high concentrations were found in the stomach.

A feature not brought out in the Tables is that the concentrations in internal organs (except the gastrointestinal tract) of rats that died were always about the same, however the rats had received the dose. In the brain the critical level was about 5 μg/g. The only exceptions were the two rats that received 185 mg/kg orally and were killed two days after injection; these

TABLE II

EXCRETION OF TIN FROM RATS GIVEN TRIPHENYLTIN CHLORIDE ORALLY

The total amount excreted is expressed as the percentage of the dose given for the following treatments:

A 185 mg/ kg in arachis oil to 2 rats
B 50 mg/ kg in arachis oil to 3 rats
C 67 mg/ kg in dimethylformamide to 3 rats. (One died 2 days after injection, so the experiment was continued with the two remaining, and amounts excreted after 2 d are expressed as percentages of the dose given to those two.)

The probable error was about 10% in every experiment.

Treatment	Time after treatment (d)								
	1	2	3	4	5	6	7	8	9
A	10	25	41	55	73	86	88	88	88
B	17	36	62	78	79	80	80	-	-
C	2	12	20	21	48	105*	110	111	111

* The high values are probably due to a sampling error on the sixth day.

rats might have died of triphenyltin poisoning had they been left longer. Their brains contained 11 μg/kg. (Table III, A). This observation is consistent with the assumption that most of the tin was present as triphenyltin and is difficult to explain in any other likely way.

TABLE III

TIN CONCENTRATIONS IN RATS TREATED ORALLY WITH TRIPHENYLTIN CHLORIDE

The column headings refer to rats treated as follows:
A Given 185 mg/kg in arachis oil and killed 2 d after injection; average of two rats.
B Same, but killed 9 d after treatment.
C Given 67 mg/kg in dimethylformamide, average of 3 rats, which died 2 d after treatment.

Tissue	Concentrations (μg triphenyltin chloride per g tissue) A	B	C
Brain	11	3.3	7.1
Liver	25	3.1	21
Kidney	22	7.5	26
Fat	9.2	0.7	7.6
Muscle	3.9	1.1	-
Stomach	331	0.6	1230
Small intestine	331	1.5	35
Large intestine	542	2.3	61

The results show that probably triphenyltin persists in the rat at appreciable concentrations for several days, and that the rat is not less susceptible than the guinea-pig to oral dosing because it decomposes triphenyltin rapidly in the gut. Under these circumstances, triphenyltin salts taken by mouth may well be very toxic to man.

A general view of the part played by tracers in the study of residues can now emerge. In the development of methods of analysis of residues in crops, tracer studies should in almost all cases be obligatory, although, of course, they are not a substitute for non-isotopic methods, which are essential for routine analysis. Tracer studies remove any doubts as to the validity of normal methods and, even in complicated cases, often give a nearly complete picture of the action of the plant on the pesticide. The work of METCALF et al. on Disyston is a particularly good example [19]. In toxicological studies tracers can often be helpful, but in most instances they are likely to yield the most useful results at an early stage in an investigation, to give way subsequently to biochemical, pharmacological and physio-

logical techniques. The main reason for this difference in utility is the difference in the relationship between a pesticide and a plant on the one hand and between a pesticide and a mammal on the other. The plant-pesticide interaction is nearly all in one direction: the plant acts on the compound, and not vice versa. Consequently when the nature, concentration and persistence of the products formed in the plant are known, the problems of interest are solved. By definition, however, in a poisoned mammal the pesticide acts on the mammal as well as the mammal on the pesticide. The problems raised by the first, toxic, action are very much more complex than those raised by the second, metabolic, action, and are not usually to be solved by labelling the pesticide.

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DISCUSSION

G.F. BURNETT (Chairman): I think we will see a good deal of the triphenyltin compounds, both as fungicides and molluscicides. Do you think we should go with care until the results of your work are fully clarified?

D.F. HEATH: Yes, unless it can be shown that the residues are in fact very low indeed. This possibility could easily be checked by using Sn^{113} -labelled triphenyltin.

C. H. SCHMIDT: Is there any evidence that triphenyltin may break down when it is applied on foliage and plants under field conditions?

D. F. HEATH: The evidence is not very good. Triphenyltin seems to break down on foliage, but break-down may be mainly due to the action of ultra-violet light. How fast triphenyltin which had run down the stalk into shady places would decompose I do not know – perhaps very slowly.

J. R. OGLE: With reference to your remarks on Demeton, there is, I think, one aspect in which the use of radioisotopes could lead to misleading results. It is a well-known fact that many dialkyl sulphides will undergo facile radiolytic oxidation to sulphoxides and sulphones. If this occurred in Demeton and related compounds in which the sulphoxides and sulphones are as toxic as the parent compound or more so, the results obtained might not be representative of the behaviour of the inactive material used under similar conditions. The rate of decomposition varies enormously from compound to compound and even between steroisomers, and is influenced by external factors such as physical state, temperature, presence or absence of moisture, oxygen, etc. It would seem that in using materials under these conditions it would be necessary to check, as in fact you did in your own work, that there was not an extra effect due to the radioisotope. For example, diisopropyl fluorophosphate-P^{32} at 40 mc/mM undergoes 10% decomposition per week but is stable in arachis oil or propylene glycol. Methionine-S^{35} at 60-100 mc/mM may undergo 20% decomposition within four weeks, depending upon the method of storage.

D. F. HEATH: In these experiments we used Demeton less than one hundredth as active as the specimens you refer to. In view of this it is perhaps not surprising that solvent distributions showed that our specimens did not break down measurably on storage. Are you normally producing pest control compounds of specific activities at which decomposition is likely to be important?

J. R. OGLE: We have recently been synthesizing insecticides at 60 to 100 mc/mM for various of our clients.

ÉVOLUTION DES DÉPÔTS SUPERFICIELS, DIFFUSION ET DÉGRADATION DE DEUX INSECTICIDES ENDOTHÉRAPIQUES: LE DÉMÉTON-S ET L'ENDOTHION DANS QUELQUES PLANTES MARAÎCHÈRES

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Abstract — Résumé — Аннотация — Resumen

CHARACTERISTICS OF SURFACE DEPOSITS, DIFFUSION AND DEGRADATION IN A NUMBER OF MARKET-GARDEN PLANTS OF TWO ENDOTHERAPIC INSECTICIDES: DEMETON-S AND ENDOTHION. In the absence of atmospheric precipitation, detoxication of plants treated with endotherapic insecticides depends on both inactivation of the surface deposits and degradation of the pesticide within the plant. Both these phenomena have been followed in a number of market-garden plants treated with demeton-S and endothion labelled with P^{32} and S^{35} respectively.

The rate of inactivation of the surface deposits is comparable as between endothion and demeton-S, where external conditions are themselves similar.

In the foliage, the persistence of demeton-S and its active metabolites varies with the plant. Normally, it is higher than that of endothion, whose disintegration pattern includes only non-toxic hydrolysis products.

In fruits treated with demeton-S, apparent degradation seems to be very rapid, whereas for endothion it remains comparable with that observed in the foliage and is apparently not affected by the maturity of the fruits. A hypothesis is put forward to explain this difference in behaviour.

Toxic residues found in the fruits may be due either to diffusion of the insecticide from the foliage or to direct contamination. A comparative study of treated and untreated fruits borne by a treated plant has made it possible to define the relative importance of these two phenomena.

Studies were made on penetration and diffusion of Demeton-S and Endothion in asparagus shoots, in an effort to explain the behaviour of these two insecticides in the control of the fly Platyparea poecilоptera. The importance of the part that may be played by the wetting agent is demonstrated.

ÉVOLUTION DES DÉPÔTS SUPERFICIELS, DIFFUSION ET DÉGRADATION DE DEUX INSECTICIDES ENDOTHÉRAPIQUES: LE DÉMÉTON-S ET L'ENDOTHION, DANS QUELQUES PLANTES MARAÎCHÈRES. En l'absence de précipitations atmosphériques, la détoxication des plantes traitées à l'aide d'insecticides endothérapiques dépend à la fois de l'inactivation des dépôts superficiels et de la dégradation du pesticide dans le végétal. Ces deux phénomènes ont été suivis sur plusieurs plantes maraîchères traitées au déméton-S et à l'endothion, marqués respectivement avec ^{32}P et ^{35}S.

La vitesse d'inactivation des dépôts superficiels est comparable pour l'endothion et le déméton-S, lorsque les conditions extérieures sont elles-mêmes voisines.

Au niveau du feuillage, la persistance du déméton-S et de ses métabolites actifs varie selon la plante traitée. Elle est en général supérieure à celle de l'endothion dont le schéma de désintégration ne comprend que des produits d'hydrolyse atoxiques.

Dans les fruits traités au déméton-S, la dégradation apparente semble très rapide; pour l'endothion, au contraire, elle reste comparable à celle que l'on observe dans le feuillage et ne semble pas affectée par la maturité des fruits. Une hypothèse est émise pour expliquer cette différence de comportement.

Les résidus toxiques retrouvés dans les fruits peuvent être dus, soit à une diffusion de l'insecticide provenant du feuillage, soit à une contamination directe. L'importance relative de ces deux phénomènes a pu être précisée grâce à une étude comparative des fruits, traités ou non, portés sur une plante elle-même traitée.

La pénétration et la diffusion du déméton-S et de l'endothion dans les pousses d'asperges sont étudiées afin de tenter d'expliquer le comportement de ces deux insecticides dans la lutte contre la mouche (Platyparea poecilоptera). Le mémoire met en évidence le rôle important que peut jouer le mouillant.

ЭВОЛЮЦИЯ ПОВЕРХНОСТНЫХ ОТЛОЖЕНИЙ, ДИФФУЗИЯ И ДЕГРАДАЦИЯ ДВУХ ЭНДОТЕРАПЕВТИЧЕСКИХ ИНСЕКТИЦИДОВ: ДЕМЕТОНА-S И ЭНДОТИОНА, В НЕКОТОРЫХ ОГОРОДНЫХ РАСТЕНИЯХ. При отсутствии атмосферных осадков детоксикация растений, обработанных с помощью эндотерапевтических инсектицидов, зависит одновременно от инактивации поверхностных отложений и от деградации пестицида в растении. Эти два феномена изучались на многих огородных растениях, обработанных деметоном-S и эндотионом, которые соответственно метились фосфором-32 и серой-35.

Скорость инактивации поверхностных отложений сходна для эндотиона и деметона-S при одинаковых внешних условиях.

На уровне листвы стойкость деметона-S и его активных метаболитов зависит от обрабатываемого растения. В основном, она выше стойкости эндотиона, схема распада которого включает лишь нездовитые продукты гидролиза.

Во фруктах, обработанных деметоном-S, видимая деградация, насколько это представляется, протекает очень быстро; и, напротив, по отношению к эндотиону она остается сравнимой с наблюдаемой в листве деградацией и не связана со зрелостью фруктов. Для объяснения этого различия подготовлена гипотеза.

Обнаруженные во фруктах ядовитые остатки могут появиться либо в результате диффузии инсектицида из листьев, либо прямого загрязнения. Сравнительное значение этих двух явлений может быть уточнено в результате сравнительного изучения обработанных или необработанных фруктов, находящихся на растении, которое также обработано.

Изучаются проникновение и диффузия деметона-S и эдотиона в побеге спаржи для объяснения способа действия этих двух инсектицидов в борьбе с мухой (Platyparea poeciloptera). Приводятся данные, показывающие важную роль влажности.

DIFUSIÓN Y DEGRADACIÓN EN ALGUNAS HORTALIZAS DE LOS DEPÓSITOS SUPERFICIALES DE DOS INSECTICIDAS ENDOTERÁPICOS: EL DEMETÓN-S Y EL ENDOTHIÓN. En ausencia de precipitaciones atmosféricas, la eliminación de los residuos tóxicos de las plantas tratadas con insecticidas endotherápicos depende a la vez de la inactivación de los depósitos superficiales y de la degradación del pesticida en el vegetal. Estos dos fenómenos han sido estudiados en varias hortalizas tratadas con Demetón-S y Endothión, marcados respectivamente con ^{32}P y ^{35}S.

Cuando las condiciones exteriores son semejantes, los depósitos superficiales del Endothión y del Demetón-S tienen una velocidad de inactivación comparable.

En el follaje, la persistencia del Demetón-S y de sus metabolitos activos varía según la planta tratada. En general, es superior a la del Endothión, cuyo esquema de desintegración sólo comprende productos de hidrólisis atóxicos.

En los frutos tratados con Demetón-S la degradación aparente parece muy rápida; en cambio, para el Endothión sigue siendo comparable a la que se observa en el follaje y no parece afectada por la madurez de los frutos. El autor de la memoria formula una hipótesis para explicar esta diferencia de comportamiento.

Los residuos tóxicos hallados en los frutos pueden deberse a una difusión del insecticida proveniente del follaje o a una contaminación directa. La importancia relativa de estos dos fenómenos se ha podido precisar gracias a un estudio comparativo de los frutos, tratados o sin tratar, dados por una planta sometida a tratamiento.

Se estudia la penetración y la difusión del Demetón-S y del Endothión en brotes de espárrago para tratar de comprender el comportamiento de estos dos insecticidas en la lucha contra la mosca (Platyparea poeciloptera). El autor de la memoria subraya la importante función que puede desempeñar el agente impregnante.

Le caractère endothérapique d'un pesticide contribue tout à la fois à l'augmentation de sa persistance et de la sélectivité de son action. Malheureusement cet avantage peut être en partie compensé par le danger dû à la présence de résidus toxiques à l'intérieur de la plante.

En effet pour les insecticides de surface, l'essentiel des résidus est constitué de dépôts extérieurs dont l'élimination peut être assurée par les facteurs climatiques: insolation, précipitations atmosphériques.

Lorsque le produit pénètre dans la plante, il se trouve de ce fait soustrait à cette action mais par contre peut servir de substrat à l'activité enzymatique du végétal.

On observe alors l'apparition de nouveaux composés dont la toxicité est très variable selon la nature du pesticide d'origine et l'évolution plus ou moins rapide selon la plante traitée.

Ainsi pour un insecticide endothérapique la contamination superficielle se trouve doublée d'une pollution interne dont l'évolution peut être complètement différente.

Enfin lorsque cet insecticide est télétoxique, les parties non atteintes directement peuvent être polluées par voie vasculaire.

Dans ce dernier cas, qui est celui du déméton-S et de l'endothion,
- la contamination superficielle,
- la contamination interne directe, et
- la contamination interne par voie vasculaire constituent trois sources de pollution dont l'importance respective et l'évolution dans le temps ne sauraient être identiques. C'est ce que nous allons étudier dans le cas particulier de quelques plantes maraîchères. Cette question revêt dans ce cas une acuité particulière, soit parce que le feuillage est consommé (salades), soit parce que la fructification étant échelonnée, il risque d'y avoir des interférences entre les traitements et les récoltes (c'est le cas en particulier des haricots cueillis en vert).

Nous envisageons ensuite quelques aspects de la pénétration et de la diffusion de ces deux insecticides dans les pousses d'asperges afin de tenter d'expliquer leur comportement dans la lutte contre Platyparea poeciloptera.

MATÉRIEL ET MÉTHODES

Plantes utilisées

L'expérimentation a porté sur les espèces suivantes: tomates, haricots nains, pois nains, et laitues. Toutes ces plantes ont été maintenues en serre tempérée pendant la totalité de leur cycle végétatif.

Produits et doses

Les insecticides choisis étaient le déméton-S et l'endothion.

L'o, o-diméthyl-thiolophosphate de S (éthyl thio 2 éthyle) ou déméton-S marqué au ^{32}P nous avait été obligeamment fourni par les établissements Bayer.

La synthèse radioactive du phosphorothiolate de ortho ortho diméthyl et de S-méthyl 2 méthoxy 5 pyrone 4 ou endothion fut réalisé au CEN de Saclay. L'élément traceur était dans ce dernier cas le ^{35}S.

Les deux insecticides ont été utilisés à une dose quatre fois supérieure à la dose efficace, soit 100 g/hl pour le demeton-S et 200 g/hl pour l'endothion.

Modalité de traitement

Etant donné la haute radioactivité spécifique de ces composés et le manque d'installation spécialisée, la pulvérisation insecticide a été remplacée par un badigeonnage de feuillage.

Lorsque cela était possible, les fruits d'un même plant ont été partagés en deux lots, l'un traité, l'autre protégé par une feuille de cellophane; ceci afin d'évaluer séparément la contamination directe (à partir de la surface traitée) et celle d'origine télétoxique.

Afin de suivre l'évolution des résidus, nous avons divisé les plantes traitées en trois lots dont la récolte était échelonnée selon le programme suivant:
déméton-S: récolte après 8, 15 et 22 j,
endothion: récolte après 5, 10 et 15 j.

La récolte des végétaux traités au déméton a été légèrement déoalée pour tenir compte de la rémanence plus grande de ce produit.

Dosage des résidus

Dans le cas du déméton-S, le dosage des résidus a été effectué selon une technique voisine de celle décrite par MÜHLMAN et TIETZ [1]:
- broyage des plantes en milieu acétonique à 80%,
- évaporation de l'acétone sous pression réduite,
- extraction chloroformique de la phase aqueuse.

La phase chloroformique ainsi obtenue contenait l'insecticide et ses dérivés toxiques (sulfoxyde et sulfone).

Dans le cas présent ce traitement était précédé d'un lavage des feuilles et des fruits traités afin d'estimer séparément l'importance des dépôts superficiels. Ce lavage a été réalisé en trois opérations successives, par agitation dans une solution de mouillant à 0,5‰. Les eaux de lavage réunies ont été soumises à une extraction chloroformique dans les mêmes conditions que précédemment.

Le degré de détoxication a été établi dans tous les cas par comparaison de la radioactivité de la phase chloroformique seule à celle du total: phase chloroformique + phase aqueuse.

L'étude de la dégradation de l'endothion a été réalisée suivant une méthode analogue; toutefois le broyage des plantes a été effectué en milieu alcoolique à 80%.

Nous signalons également que pour ce produit, seul l'insecticide non décomposé passe dans la phase chloroformique.

ÉVOLUTION DES DÉPÔTS SUPERFICIELS (tableau I)

Comme nous l'avons signalé, la dégradation du film insecticide déposé à la surface de la plante dépend essentiellement des conditions climatiques. Cependant la feuille ne saurait être considérée comme un support inerte puisqu'elle est le centre d'échanges actifs entre la plante et l'atmosphère. D'autre part la texture de la surface foliaire, tomenteuse ou lisse, varie d'une plante à l'autre; chez une même plante des différences analogues peuvent également être observées entre feuilles et fruits (tomates par exemple). On pouvait donc espérer quelques variations entre les vitesses de dégradations mesurées à la surface des différentes plantes expérimentées.

Or, pour un traitement réalisé dans des conditions extérieures identiques (série A), les différences constatées sont extrêmement réduites; qu'il

TABLEAU I

ÉVOLUTION DES DÉPÔTS SUPERFICIELS* EN FONCTION DU TEMPS

	Plantes traitées		Temps écoulé entre le traitement et la récolte				
			5 j	8 j	10 j	15 j	22 j
ENDOTHION							
Série A	Tomates	Feuilles	71		47,6	35,3	
		Fruits	74,9		60	46,8	
	Haricots	Feuilles	69,9		58	35,9	
		Fruits	67,5		63	47,8	
	Petits Pois	Feuilles	73,2		55	30	
		Fruits	56		49,5	21,3	
Série B	Tomates	Feuilles	78		66,3	54,2	
		Fruits	76,1		61	43	
	Haricots	Feuilles	88,2		70,6	42,2	
		Fruits	86,4		74,4	54	
DÉMÉTON-S							
	Tomates	Feuilles et fruits		57		49,3	31,2
	Salades	Feuilles		68,2		55	42,3

* Pourcentage de la radioactivité présente dans la phase chloroformique

s'agisse de surfaces foliaires d'espèces différentes ou bien que la comparaison porte sur les feuilles et les fruits d'une même espèce.

Dans nos conditions expérimentales, la nature de la surface traitée (feuille ou fruit) est donc sans influence pratique sur la vitesse de dégradation sauf peut-être chez le pois où l'on note une décomposition accrue à la surface du fruit.

Il y a une seule exception, semble-t-il: la disparition des résidus de déméton-S paraît retardée sur les feuilles de salade; ceci s'explique dans une certaine mesure par la disposition particulière du feuillage de cette plante qui protège l'insecticide de l'action des agents extérieurs.

Si nous comparons maintenant les vitesses de dégradation respectives des deux insecticides expérimentés, on constate que, pour une même plante, la tomate, les dépôts superficiels d'endothion sont un peu moins tenaces que ne le sont les métabolites actifs du déméton-S.

En l'absence de certaines conditions extérieures, pluie abondante ou insolation intense, la détoxication des dépôts superficiels d'endothion ou

de déméton-S semble assez lente. Après 15 jours il reste encore 20 à 50% de l'endothion et 50 à 55% du déméton-S; après trois semaines 30-40% du déméton-S sont encore présent sur le feuillage.

RÉMANENCE DES RÉSIDUS TOXIQUES DANS LE FEUILLAGE DES PLANTES TRAITÉES (tableau II)

La persistance de la toxicité chez les plantes traitées dépend avant tout de la nature chimique du pesticide qui conditionne l'existence et la nature de métabolites toxiques éventuels.

Or, nous l'avons déjà signalé, le schéma de dégradation de l'endothion est totalement différent de celui du déméton-S et ne comprend que des produits d'hydrolyse atoxiques; ceci se traduit pratiquement par une rémanence moins accentuée. C'est ce que l'on peut constater en comparant les résultats obtenus sur tomates pour ces deux insecticides. 15 jours après le traitement le déméton-S et ses métabolites toxiques constituent encore 66% des résidus phosphorés présents dans la plante tandis que 90% environ de l'endothion a déjà disparu.

TABLEAU II

RÉMANENCE* DE L'ENDOTHION ET DU DÉMÉTON-S DANS LES PLANTES TRAITÉES

	Plantes traitées	Temps écoulé entre le traitement et la récolte				
		5 j	8 j	10 j	15 j	22j
ENDOTHION						
Feuilles (série A)	Tomates	41,9		17,6	11,8	
	Haricots	25		21,8	13,3	
	Pois	45		38	14,2	
Feuilles (moyennes)	Tomates	52,6		13,8	12	
	Haricots	39,7		20,5	12	
Fruits (moyennes)	Tomates	58,1		32,6	12,9	
	Haricots	34,6		24,1	7,9	
DÉMÉTON-S						
Feuilles	Laitue		91,2		86,5	83,7
	Tomate		89,7		66,3	42,7
Fruits	Tomate		34		14	4 4

* Pourcentage de la radioactivité présente dans la phase chloroformique

La nature de la plante traitée peut elle aussi, affecter la vitesse de dégradation d'un insecticide; BENNETT et THOMAS ont démontré que le schradan se décompose plus rapidement chez le haricot que dans le chrysanthème ou le coleus [2]. METCALF et autres ont constaté pour le disyston une métabolisation accélérée chez la tomate [3]. Une observation analogue peut être faite pour le déméton-S et l'endothion.

Appliqué sur les feuilles de salades, le déméton-S se dégrade très lentement. 21 jours après le traitement le pourcentage de destruction ne dépasse pas 16%; durant le même temps il atteint 53% chez la tomate.

Pour l'endothion, la vitesse de métabolisation est à peu près la même chez la tomate et le petit pois, mais plus élévée chez le haricot.

PERSISTANCE DES RÉSIDUS TOXIQUES DANS LES FRUITS

En premier lieu, il convenait d'établir si la disparition des résidus toxiques était aussi rapide dans les fruits que dans les feuilles. Ce problème était important car pour trois des plantes étudiées le fruit constituait la partie consommable de la plante.

Il semble y avoir sur ce point précis une différence notable entre l'endothion et le déméton-S.

Chez les plantes traitées à l'endothion, la rapidité de la dégradation semble voisine dans le feuillage et les fruits traités.

Dans les fruits non traités l'évolution des résidus toxiques est comparable pendant les premiers jours (tableau III, série C). On assiste ensuite à une détoxication extrêmement rapide.

Pour le déméton-S, la vitesse apparente de détoxication s'accentue immédiatement quand on passe des feuilles aux fruits traités; elle atteint son maximum dans les fruits n'ayant reçu aucun traitement. Après 14 jours la proportion de résidus toxiques est la suivante:

Feuilles: 66%
Fruits traités: 14%
Fruits non traités: 7%.

TABLEAU III

VITESSE DE DÉGRADATION* DES DEUX PESTICIDES DANS LES DIFFÉRENTES PARTIES DU VÉGÉTAL

Temps écoulé entre le traitement et la récolte	Endothion (série C)			Déméton-S	
	5 j	10 j	15 j	8 j	15 j
Feuilles traitées	66	10	7,3	89,7	66,3
Fruits traités	59	32,6	19,6	34	14
Fruits non traités	73	3,4	0,66	21	7

* Pourcentage de la radioactivité présente dans la phase chloroformique

La feuille et le fruit étant des organes dont le métabolisme est différent, on conçoit fort bien que les vitesses de destruction d'un insecticide y soient elles-mêmes différentes.

Ce qui semble moins évident est la disparité que l'on constate entre fruits traités et non traités. Que l'insecticide parvienne par voie vasculaire ou à partir de l'épiderme ne change rien à sa vitesse de dégradation.

On est donc conduit à admettre que ce qui parvient au fruit par la voie vasculaire est surtout composé de produits d'hydrolyse voire même de restes phosphorés ou soufrés réutilisés par la plante et drainés vers les centres d'activité métabolique que constituent les fruits en croissance.

Pour le déméton-S ce transport de métabolites non extractibles par le chloroforme doit commencer très tôt, ce qui expliquerait la faible proportion de résidus toxiques retrouvée dès le septième jour et l'accentuation du phénomène dans les fruits non traités (voir tableau III).

Au contraire, c'est l'endothion qui constitue l'essentiel des substances soufrées qui parviennent aux fruits durant les cinq premiers jours qui suivent le traitement; puis l'insecticide est rapidement remplacé par des dérivés atoxiques et dénués de pouvoir insecticide.

Comparons maintenant, pour chacun des insecticides, leurs vitesses de dégradation à la surface et à l'intérieur de la plante traitée.

Pour le déméton-S, au niveau du feuillage, c'est le film superficiel qui se dégrade le plus rapidement; au niveau des fruits, c'est la pollution interne qui disparaît la première.

En ce qui concerne l'endothion dans nos conditions expérimentales, la dégradation est toujours plus rapide à l'intérieur de la plante qu'à l'extérieur.

Dans l'une des séries expérimentales nous avons étudié séparément la persistance de l'endothion dans les fruits mûrs ou encore verts.

Au 5^{e} jour la détoxication s'est révélée beaucoup plus grande dans les fruits verts. Ce fait pouvant présenter une certaine importance au point de vue toxicologie, nous avons repris l'étude sur des broyats de fruits réalisés dans divers tampons allant de pH 6 à pH 8. Dans toutes les expériences réalisées le taux de dégradation du pesticide était très voisin pour les deux types de fruits.

IMPORTANCE RELATIVE DES DÉPÔTS SUPERFICIELS, DE LA CONTAMINATION DIRECTE ET DU TRANSPORT INTERNE DANS LA POLLUTION DES PLANTES TRAITÉES

Les modalités de la dénaturation du pesticide ne constitue évidemment qu'une donnée dans le problème des résidus. Nous allons maintenant en aborder l'aspect quantitatif. La technique de traitement que nous avons dû adopter pour des raisons de sécurité s'est révélée assez imparfaite et il existe une irrégularité évidente entre les dépôts. Les comparaisons qui vont suivre ont donc été établies sur les moyennes de trois traitements.

Feuillage

Les dépôts foliaires d'endothion sont très importants, 150 à 270 ppm, soit environ 15 à 27 γ/cm^2 (à peu près quatre fois la dose normale). Cette

saturation a été recherchée afin d'accentuer au maximum la pénétration du produit. Dans ces conditions on retrouve dans le feuillage de 60 à 120 ppm de produits marqués.

Ceci est sans intérêt pour les tomates et les haricots dont le feuillage n'est pas consommé, mais on doit en tenir compte dans le cas des pois car le feuillage de cette plante est parfois utilisé (après cueillette en vert et battage mécanique) pour la nourriture du bétail. Etant donné qu'une pulvérisation normale réduirait au quart les résidus laissés à la surface et dans la plante, la contamination quinze jours après le traitement serait la suivante:

Dépôts superficiels: $\frac{153,8}{4} = 40$ ppm $\times$ 33,3 = 13 ppm

Résidus internes : $\frac{121,2}{4} = 30$ ppm $\times$ 14,2 = 4 ppm

Même si l'on envisage une réduction importante de la pollution superficielle par les intempéries, la quantité de pesticide présente dans le tissu foliaire rend celui-ci dangereux pour l'alimentation du bétail deux semaines après le traitement.

Les quantités de déméton-S déposées à la surface des deux plantes expérimentées sont assez réduites et correspondent sensiblement à l'apport d'une application normale. Pour la tomate le coefficient de pénétration est toujours sensiblement plus élevé que celui de l'endothion, mais ceci peut être une conséquence du caractère limité de l'application. Il atteint une valeur très grande lorsque le traitement est réalisé sur les salades. Un coefficient de pénétration aussi important joint à une détoxication très lente chez ce végétal rend impropre l'emploi de cet insecticide dans ce cas particulier.

Fruits

La teneur en résidus des fruits traités dépend à la fois de la contamination directe à partir de la surface et de l'apport interne, conséquence du caractère télétoxique des pesticides.

Il est évidemment difficile d'avoir une idée exacte de l'importance relative de ces deux apports.

Afin d'obtenir un ordre de grandeur, nous avons utilisé un artifice expérimental en abritant un fruit sur deux lorsque ceux-ci étaient voisins et de préférence disposés sur la même « grappe ». Dans ces conditions nous avons admis que l'apport interne était comparable pour l'un et l'autre fruit, compte tenu évidemment de leur masse respective. Ceci n'est d'ailleurs qu'une approximation, car la répartition d'un pesticide dans une plante est largement hétérogène.

Ces conventions étant admises, la comparaison des résultats portés dans les colonne 6 et 7 du tableau IV nous permet de juger l'importance de l'apport vasculaire dans la contamination interne des fruits traités.

On constate que la totalité des substances marquées, drainées vers les fruits, pourrait, si elle était constituée uniquement de métabolites toxiques, assurer 50 à 70% de leur contamination interne. En fait, on s'aperçoit que la pollution réelle due à l'effet télétoxique va en s'amenuisant ra-

pidement (tableau IV). Après 15 jours, elle peut être tenue pour négligeable pour toutes les plantes étudiées, qu'il s'agisse de l'endothion ou du déméton.

Il n'en est pas de même pour la contamination externe. Dans les conditions de nos essais, les dépôts superficiels constituent parfois la source de pollution essentielle des fruits traités. Nous avons pu l'établir pour l'endothion. Quelle que soit la plante que nous ayons traitée, cet insecticide persistait toujours plus longtemps à la surface qu'à l'intérieur du fruit; l'importance relative de la contamination externe croît donc avec le temps.

Ainsi chez la tomate elle représente 83% de la pollution totale au 5^{e} jour et 91% après 15 jours.

En ce qui concerne le déméton, l'évolution est encore plus nette; toujours chez la tomate, la contamination externe assure 54% de la contamination totale au quinzième jour et 76% au vingt-et-unième jour.

Quel que soit d'ailleurs le pesticide employé, la quantité de résidus retrouvée dans les fruits de tomate paraît réduite, surtout si nous la comparons à celle présente dans les gousses de haricots et de pois (voir tableau IV, colonnes 8 et 9).

Ceci est lié, d'une part, à la faible contamination superficielle, due elle-même à la surface lisse de ce fruit et aussi à la dilution que subit l'apport interne; chez la tomate, les fruits constituaient, dans nos essais, une masse beaucoup plus importante que les feuilles (500 à 600%).

Inversement, la pollution élevée des gousses de pois et de haricots s'explique autant par une lourde contamination superficielle que par un apport interne notable dû à la masse assez réduite que les fruits représentent par rapport aux feuilles (voir tableau IV, colonne 14).

PÉNÉTRATION ET DIFFUSION DU DÉMÉTON-S ET DE L'ENDOTHION DANS LES POUSSES D'ASPERGES

A la suite d'essais réalisés dans le cadre de la lutte contre la mouche de l'asperge (Platyparea poeciloptera), une différence d'efficacité avait été remarquée en faveur de l'endothion.

En dehors de toute spécificité vis-à-vis de l'insecte lui-même, nous avons tenté de vérifier si l'efficacité de l'endothion ne pouvait être expliquée par une meilleure pénétration à travers l'écorce ou une diffusion plus importante dans la pousse d'asperge.

Dans ce but deux types d'expérimentations ont été réalisés:

1° Essais de «pénétration»: toute la surface de la pousse étant traitée, nous avons évalué:
   a) la pénétration dans la plante,
   b) la diffusion de la zone corticale vers le centre.

2° Essais de «diffusion»: dans ce type d'expérimentation seule la partie médiane de la pousse recevait l'insecticide. Nous avons ensuite suivi le passage de ce composé:
   a) vers le centre,
   b) vers les extrémités apicales et basales.

TABLEAU IV

IMPORTANCE RELATIVE DES DÉPÔTS SUPERFICIELS, DE LA CONTAMINATION DIRECTE ET DU TRANSPORT INTERNE DANS LA POLLUTION DES PLANTES TRAITÉES

		FEUILLAGE TRAITÉ				FRUITS TRAITÉS				FRUITS NON TRAITÉS			
		Contamination externe		Contamination interne	I/T(1)	Contamination externe		Contamination interne	I/T	(Effet télétoxique)		ET/I(3)	Poids des fruits / Poids des feuilles
		RT(2)	E.C.(5)	RT E.C.	(%)	RT	E.C.	RT E.C.	(%)	RT	E.C.	(%)	(%)
ENDOTHION (série A)													
	5 j		149(4)	34	18		2,4	0,5	17		0,22	30	
Tomates		210(4)		81		3,2		1,25		0,9			608
	15 j		74	13	15		1,5	0,15	9		0,02	11	
	5 j		188	14	7		8,4	3,3	28		0,62	28	
Haricots		268		58		12,5		11,7		6,2			180
	15 j		95	11	10		6	1,1	15		0,02	18	
	5 j		112	54	32		5,1	3,5	40		1,4	28	
Pois		154		121		9,2		12,3		9			92
	15 j		51	46	47		1,9				0,01		
DÉMÉTON-S													
	15 j		4,75	8,4	64		0,45	0,39	46		0,012	3,1	
Tomates		9,6		12,7		0,92		2,48		1,8			
	21 j		3,97	5,4	57		0,38	0,12	24		0,017	14	
	15 j		1,65	9,7	85								
Salades		3		11,2									
	21 j		1,27	9,4	88								

(1) - I/T: contamination interne/contamination totale.
(2) - RT: radioactivité totale exprimée en équivalent insecticide.
(3) - ET/I: effet télétoxique/contamination interne des fruits traités.
(4) - concentrations exprimées en ppm.
(5) - E.C.: extrait chloroformique.

De plus, pour le déméton seulement, nous avons complété ce travail en étudiant:
a) l'influence de l'addition d'un émulsifiant,
b) l'efficacité comparée de 2 émulsifiants (formule A et B).
L'endothion a toujours été utilisé avec l'adjuvant A.

Les modes opératoires étaient les suivants:
- l'expérimentation a été réalisée en serre,
- les traitements et l'évaluation des résidus ont été effectués selon les méthodes décrites dans l'essai précédent,
- les deux insecticides ont été utilisés à la dose de 1 mg/cc soit 100 g/hl,
- la durée de l'essai était de 48 h.

Résultats obtenus

1. Pénétration dans la plante (voir tableau V)

a) Influence de l'émulsifiant (déméton seulement)

L'hétérogénéité des résultats de la série «sans émulsifiant» ne permet pas de conclure avec certitude. Il semble toutefois que l'adjuvant utilisé accroît la concentration de l'insecticide dans les plantes. La teneur importante (2,4 ppm) constatée dans un lot d'asperges traitées en l'absence d'adjuvant pourrait s'expliquer ainsi: ce lot étant constitué de pousses de faible section, le rapport surface/volume se trouvait donc augmenté, ce qui a entraîné un accroissement du taux de pénétration apparente.

TABLEAU V

PÉNÉTRATION DE L'ENDOTHION ET DU DÉMÉTON-S A TRAVERS L'ÉCORCE DES POUSSES D'ASPERGES.

	Poids(1) (g)	Conc.(2) (ppm)	Poids (g)	Conc. (ppm)
DÉMÉTON-S				
Emulsifiant B	20,3	3,9	29,7	2,6
Emulsifiant A	28,2	1,93	19,5	2,2
Sans émulsifiant	25,3	0,77	12,2	2,4
ENDOTHION				
Emulsifiant A	28,2	5,9	12,2	38,6

(1) Poids moyen de la partie traitée
(2) Concentration en insecticide (radioactivité totale mesurée dans la pousse, exprimée en équivalent d'insecticide)

b) Influence de la nature de l'insecticide.

L'endothion semble pénétrer en quantité beaucoup plus importante que ne le fait le déméton. Qu'il s'agisse des essais de «pénétration» ou de «diffusion», les teneurs élevées en insecticide sont toujours en faveur de l'endothion.

TABLEAU VI

DIFFUSION DE L'ENDOTHION ET DU DÉMÉTON-S VERS LA ZONE CENTRALE DE L'ASPERGE

	Teneur en insecticide*		Taux de diffusion (%)
	Ecorce	Centre	
DÉMÉTON-S			
Emulsifiant B	45,72	6,44	12,3
Emulsifiant A	30,39	3,75	11
Sans émulsifiant	34,35	8,59	20
ENDOTHION			
Emulsifiant A	116,00	36,36	23,8

* Radioactivité totale exprimé en équivalent insecticide.

2. Diffusion vers le centre de la pousse d'asperge (voir tableau VI)

a) Influence du mouillant (déméton seulement).

L'addition de l'un ou de l'autre des adjuvants expérimentés influence défavorablement la diffusion vers la zone centrale, comme on peut le constater dans le tableau VI.

b) Influence de la nature de l'insecticide.

Le même tableau met en évidence une diffusion plus marquée de l'endothion vers le centre de la pousse d'asperge.

3. Diffusion vers les extrémités non traitées (voir tableau VII)

a) Influence du mouillant (déméton seulement).

Les résultats sont comparables à ceux que nous avons observé pour la diffusion vers la partie centrale. C'est le déméton exempt d'adjuvant qui migre en quantité la plus importante.

La diffusion est d'ailleurs essentiellement dirigée vers le haut. La partie basale de l'asperge ne reçoit pratiquement rien.

TABLEAU VII

DIFFUSION DE L'ENDOTHION ET DU DÉMÉTON-S VERS LES EXTRÉMITÉS NON TRAITÉES.

	Teneur en insecticide			Taux de diffusion * (%)	
	Base non traitée (ppm)	Partie traitée (ppm)	Haut non traitée (ppm)	haut	bas
DÉMÉTON-S					
Emulsifiant B	0,02	0,78	0,72	36,7	1,6
Emulsifiant A	0,06	0,72	0,77	40,2	5,4
Sans émulsifiant	0,09	0,47	0,68	38,2	5,4
ENDOTHION					
Emulsifiant A		6,13	2,32	17,4	

* Etabli sur les teneurs mesurées en valeur absolue.

b) Influence de la nature de l'insecticide

Dans la partie haute de la pousse, la teneur en endothion se montre toujours supérieure à la teneur en déméton. Ceci est simplement dû à la concentration plus forte de l'endothion dans la totalité de la plante, car dans le cas présent c'est le déméton qui possède le taux de diffusion le plus élevé.

L'endothion, comme le déméton, ne migre pratiquement pas vers la base des pousses.

4. Dégradation des insecticides dans les différentes parties de la pousse (voir tableau VIII)

Il convenait de s'assurer que les molécules marquées sillonnant la plante correspondaient effectivement à des produits actifs et non à des métabolites sans intérêt sur le plan insecticide. Ceci nous a amenés à suivre au cours de l'essai précédent les taux de dégradation dans les différentes parties de la pousse d'asperge.

Pour le déméton le taux de dégradation reste assez faible mais croît malgré tout de l'écorce traitée vers l'intérieur et les extrémités. Dans le cas de l'endothion le taux de destruction de l'insecticide paraît légèrement plus élevé, mais il ne semble pas que la migration du produit s'accompagne d'une dégradation plus accentuée.

En résumé la nature de l'émulsifiant stabilisateur ne semble pas modifier de façon notable la pénétration ou la diffusion du déméton. L'action la plus nette des deux formules d'adjuvants serait de réduire légèrement la diffusion de l'insecticide.

TABLEAU VIII

DÉGRADATION* DES DEUX INSECTICIDES DANS LES DIFFÉRENTES PARTIES DE L'ASPERGE

	Partie traitée		Haut non traité
	Ecorce	Centre	Total
Essai «diffusion»			
DÉMÉTON-S			
Emulsifiant B	78,5	49,7	58,5
Emulsifiant A	72,5	68	59,5
Sans émulsifiant	92,7	67,5	76,7
ENDOTHION			
Emulsifiant A	64	58,5	60
Essai «pénétration»			
DÉMÉTON-S			
Emulsifiant B	86,3	76,9	
Emulsifiant A	80,6	63,5	
Sans émulsifiant	83,9	86,9	

* Dégradation exprimé en pourcentage de la radioactivité présente dans la phase chloroformique

En dehors de toute spécificité vis-à-vis de l'insecte l'efficacité accrue de l'endothion pourrait donc s'expliquer par une concentration plus élevée dans les pousses d'asperges.

CONCLUSION

Nous avons étudié le problème des résidus toxiques laissés dans quelques plantes maraîchères par deux insecticides endothérapiques, le déméton-S et l'endothion.

En dehors de toute précipitation atmosphérique, l'importance relative des dépôts superficiels et de la contamination interne évolue de façon différente selon l'insecticide, la plante, et pour une même plante selon qu'il s'agit des feuilles ou des fruits.

La voie vasculaire peut assurer une part importante de la contamination des fruits lorsqu'on utilise l'endothion.

Par contre ce type de pollution reste tout à fait secondaire dans le cas du déméton.

Quinze jours après le traitement l'effet télétoxique de ces deux insecticides semble sans influence pratique sur le taux de contamination interne des fruits traités.

Dans les conditions de nos essais, la contamination interne décroît très lentement chez les salades traitées au déméton.

Par contre quel que soit l'insecticide utilisé, la pollution externe et interne des fruits de tomate reste faible.

La persistance de l'endothion dans les plantes traitées est inférieure à celle du déméton-S.

L'efficacité plus grande de l'endothion dans la lutte contre la mouche de l'asperge (Platyparea poeciloptera) pourrait s'expliquer par une meilleure pénétration dans la pousse d'asperge.

L'absence de diffusion vers l'extrémité basale de la pousse, qui est l'endroit où les larves du 3^{e} âge creusent leurs galeries, serait également en accord avec l'inefficacité des deux insecticides contre les larves ayant atteint ce stade.

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DISCUSSION

J.E. CASIDA: Did you subject any of your extracts to chromatographic studies?

M. HASCOËT: In view of the large number of extractions which this work required it was not possible for us to carry out a chromatographic test in every case. We did, however, carry out this study for endothion, where only the non-degraded insecticide passes into the chloroform phase. Endothion has three degradation products. One of them, a monomethylated product, is now known. The two others we have not yet been able to identify exactly. One of them does not contain any phosphorus; they are probably hydrolysis products but they do not correspond to the products obtained by normal chemical hydrolysis.

Although these were the only degradation products we subjected to a chromatographic test, each insecticide was given a chromatographic test for chemical purity before being used.

L'ÉTABLISSEMENT DES PROCESSUS D'ABSORPTION ET DIFFUSION DES INSECTICIDES SYSTÉMIQUES AU POPULUS x EURAMERICANA DODE GUINIER «ROBUSTA»

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Abstract — Résumé — Аннотация — Resumen

ESTABLISHMENT OF THE PROCESSES OF ABSORPTION AND DIFFUSION OF SYSTEMIC INSECTICIDES IN POPULUS EURAMERICANA DODE GUINIER "ROBUSTA". The organophosphoric insecticides having systemic properties due to their ability to penetrate into the sap flow of plants act on insects which spend part of their lives either in the cambial region between wood and bark, or in the wood itself. Until the introduction of these new insecticides chemical control of xylophagous insects in tree-trunks was almost impossible.

The mechanism of absorption, diffusion and concentration of systemic insecticides in species of poplar and willow, which are frequently attacked by xylophagous insects, was studied using labelled Dipterex on the poplar Robusta R20.

The insecticide was labelled in the reactor, using powdered Dipterex (1.5 g) as target, at a flux $\Phi = 10^{11}$ n/cm² s and at a temperature of 30-40°C. Irradiation was carried on until an absolute target activity of ≅1 mc was obtained.

The insecticide was administered, in the form of a solution, by pouring it on to the soil, except in one case in which the insecticide was sprayed on to the leaves.

One series of experiments was conducted in the laboratory using seedlings grown in pots, and another series was perfonned in a nursery, using one- and two-year-old saplings.

One to two months after administration of the labelled insecticide solutions the trees were subjected to radiometric analysis.

In the laboratory experiments the insecticide accumulated in considerable quantities in the leaves, and in smaller amounts in the wood.

Both in the laboratory and in the nursery a greater amount of insecticide accumulated in the leaves. In the nursery experiment, however, a considerable increase was observed in the accumulation in the branches and in the wood of the stem, especially at the extremities.

The mean accumulation of insecticide amounted to 1.65 mg/g green matter in the nursery as against 0.24 mg/g in pots.

It was shown that under field conditions, in dry weather, two-year-old Robusta saplings take up Dipterex administered in an aqueous solution at the rate of 9.3% of the amount applied to the oil, as compared with 1.35% in laboratory experiments.

Application of solution to the leaves by spraying yields a low insecticide absorption rate owing both to the difficult process of uptake through the leaves and to the removal of the insecticide by rain, dew and mist.

Much insecticide was found on the ground and the authors therefore concluded that the method of administering systemic insecticide solutions through the soil should be adopted as a priority measure in the campaign against tree-species pests.

L'ÉTABLISSEMENT DES PROCESSUS D'ABSORPTION ET DIFFUSION DES INSECTICIDES SYSTÉMIQUES AU POPULUS X EURAMERICANA DODE GUINIER «ROBUSTA» Les insecticides organophosphoriques à propriétés systémiques, par leur faculté de pénétrer dans le courant de sève des plantes, ont une action sur les insectes qui mènent une partie de leur vie soit dans la zone cambiale entre le bois et l'écorce, soit dans le bois. Jusqu'à l'apparition de ces nouveaux insecticides, la lutte chimique contre les insectes xylophages de la tige des arbres était presque impossible.

Pour étudier le mécanisme d'absorption, de diffusion et de localisation des insecticides systémiques en ce qui concerne les peupliers et les saules, espèces fréquemment attaquées par les insectes xylophages, on a fait des recherches en employant le «Dipterex» marqué sur le peuplier Robusta R20.

Le marquage de l'insecticide a été fait dans un réacteur, en utilisant comme cible le Dipterex en poudre (1,5 g), avec un flux $\Phi = 10^{11}$ n/cm^2 · s et à une température de 30 à 40°C. L'irradiation a été effectuée jusqu'à l'obtention d'une activité absolue de la cible ≅ 1 mc.

L'administration de l'insecticide a été faite par verse des solutions dans le sol, sauf dans un cas où l'on a pulvérisé l'insecticide sur l'appareil foliacé.

Une série d'expériences a été faite en laboratoire, en utilisant des plants cultivés en pots; une autre série a été faite, en pépinière, avec des plants de un et deux ans.

En un laps de temps de un à deux mois après l'administration des solutions d'insecticide marqué, les plants ont été soumis à l'analyse radiométrique.

Dans les expériences en laboratoire, l'insecticide a été accumulé en grandes quantités dans les feuilles et en plus petites quantités dans le bois.

Tant en laboratoire qu'en pépinière, l'insecticide a été accumulé en plus grande proportion dans les feuilles. Pourtant, on constate une augmentation importante des accumulations d'insecticide dans les rameaux et dans le bois de la tige, surtout au bout des plants, dans l'expérience en pépinière.

En général, l'accumulation d'insecticide a été de 1,65 mg/g substance verte en pépinière, contre 0,24 mg/g en pots.

Il en résulte que, dans les conditions de terrain, les plants de peuplier Robusta de 2 ans, pendant le temps sec, peuvent mobiliser le Dipterex administré en solution aqueuse à raison de 9,3% de la quantité versée dans le sol, contre 1,35% dans les expériences en laboratoire.

L'administration des solutions sur les feuilles par pulvérisation a un rendement faible d'absorption de l'insecticide, grâce aussi bien au mécanisme difficile d'absorption des feuilles qu'au lavage de l'insecticide par l'eau de pluie, la rosée et le brouillard.

On a décelé une grande quantité d'insecticide sur le sol; c'est pourquoi les auteurs considèrent que la méthode d'administration des solutions d'insecticides systémiques dans le sol doit être adoptée en priorité dans la lutte contre les ennemis des essences ligneuses.

ОПРЕДЕЛЕНИЕ ПРОЦЕССОВ ПОГЛОЩЕНИЯ И ДИФФУЗИИ СОМАТИЧЕСКИХ ИНСЕКТИЦИДОВ У POPULUS X EURAMERICANA DODE GUINIER "ROBUSTA". Фосфорооргапические инсектициды с соматическими свойствами, связанными с их способностью проникать в сок растений, воздействуют на насекомых, которые проводят часть своей жизни либо в камбиальной зоне между древесиной и корой, либо в древесине. До появления этих инсектицидов химическая борьба против ксилофаговых насекомых, находящихся в стволе растений, была почти невозможной.

Для изучения механизма поглощения, диффузии и локализации соматических инсектицидов у тополя и ивы, которые часто подвергаются нападению ксилофаговых насекомых, были проведены исследования с применением меченого "Dipterex" на тополе Robusta R-20.

Мечение инсектицида было произведено в реакторе с использованием в качестве мишени "Dipterex" в порошке (1,5 г) при потоке в $\Phi = 10^{11}$ н/см^{2}сек и при температуре в 30 - 40°С. Это продолжалось до получения абсолютной активности мишени ≈ 1 мкк.

Введение инсектицида производилось путем полива земли, за исключением одного эксперимента, когда инсектицид разбрызгивался на листья.

Часть опытов проводилась в лабораториях, где использовались растения, выращенные в вегетационных сосудах. Другая часть опытов проводилась на почве, в питомниках, с использованием одно- летних и двухлетних растений.

В течение 1 - 2 месяцев после введения растворов меченого инсектицида растения подвергались радиометрическому анализу.

При проведении опытов в лабораторных условиях инсектицид накапливался в значительных количествах в листве и в незначительных количествах - в древесине.

При проведении опытов как в лабораторных условиях, так и на почве инсектицид накапливался в большем процентном отношении в листве. Вместе с тем отмечается значительный рост накоплений инсектицида в ветвях и в древесине ствола, особенно в концевых частях растений.

В общем, при данном опыте накопление инсектицида составило 1,65 мг/г зеленого вещества по сравнению с 0,24 мг/г этого же вещества у растений, выращенных в лабораторных условиях.

Из этого следует, что на почве двухлетние тополи Robusta могут в сухое время года накапливать "Dipterex", который был введен в водном растворе в отношении 9,3% к количеству, внесенному в землю. При проведении опытов в лабораторных условиях это отношение составляло 1,35%.

Нанесение растворов на листву путем разбрызгивания дает слабый коэффициент поглощения ин-

сектицида, что объясняется также трудным процессом поглощения у листьев и смыванием инсектицида дождевой водой, росой и туманом.

На земле обнаружено большое количество инсектицида. В связи с этим мы считаем, что методу внесения растворов соматических инсектицидов в почву должно быть отдано предпочтение в борьбе с вредителями древесных пород.

DETERMINACIÓN DE LOS PROCESOS DE ABSORCIÓN Y DIFUSIÓN DE LOS INSECTICIDAS SISTÉMICOS EN EL POPULUS X EURAMERICANA DODE GUINIER «ROBUSTA». Los insecticidas organofosfóricos de acción indirecta penetran en la corriente de savia de las plantas y actúan así sobre los insectos que viven en el leño o en la zona cambial situada entre el leño y la corteza. Hasta la aparición de estos nuevos insecticidas era casi imposible luchar por medios químicos contra los insectos xilófagos del tronco de los árboles.

Para estudiar el mecanismo de absorción, difusión y localización de los insecticidas de acción indirecta en las distintas especies de álamos y sauces que suelen verse atacados por insectos xilófagos, se han hecho investigaciones con el álamo Robusta R 20 empleando «Dipterex» marcado.

La marcación del insecticida se hizo en un reactor, sometiendo el Dipterex en polvo (1,5 g) a un flujo $\Phi = 10^{11}$ n/cm^2·s, a una temperatura de 30-40°C. Se prosiguió la irradiación hasta obtener una actividad absoluta de ≈1 mc.

Salvo en un experimento, en el que se pulverizó el insecticida en el follaje, en todos los demás se regó el suelo con soluciones insecticidas.

Parte de los experimentos se efectuaron en laboratorio, con plántulas cultivadas en macetas, y los demás en semillero y en las condiciones normales del terreno, con plántulas de uno y dos años.

Un mes o dos después de haber administrado las soluciones de insecticida marcado se realizó el análisis radiométrico de las plántulas.

En los experimentos de laboratorio, el insecticida se había acumulado en grandes cantidades en las hojas y en cantidades más pequeñas en el leño.

En los experimentos hechos en semillero y en las condiciones normales de terreno, el insecticida se acumuló en mayor proporción en las hojas, pero se pudo observar un aumento importante de la cantidad de insecticida acumulado en las ramas y en el leño del tronco, en especial en las extremidades de las plántulas.

Por regla general, en estos experimentos la acumulación de insecticida ha sido de 1,65 mg/g de sustancia verde, mientras que en las plántulas cultivadas en laboratorio fue de 0,24 mg/g.

De ello se desprende que en las condiciones normales de terreno, las plántulas de álamo Robusta de dos años pueden movilizar durante la estación seca el 9,3% del Dipterex vertido en el suelo en solución acuosa, mientras que en los experimentos de laboratorio sólo movilizan el 1,35%.

Si la solución se pulveriza en las hojas, la cantidad de insecticida absorbido es relativamente baja, debido a que la absorción se produce difícilmente en las hojas y a que la lluvia, el rocío y la niebla arrastran al insecticida.

Como se ha observado que en el suelo queda gran cantidad de insecticida, los autores consideran que en la lucha contra los insectos que atacan a las especies leñosas es preferible verter en el suelo las soluciones de insecticidas de acción indirecta.

1. INTRODUCTION

Par leur propriété de s'intégrer dans le courant de sève des plantes, les insecticides organophosphoriques à propriétés systémiques immunisent, d'une part, les plantes aux attaques des insectes nuisibles et, d'autre part, détruisent les insectes nuisibles dans les organes divers de la plante. La fonction systémique assure la pénétration de l'insecticide dans les tissus vivants, évitant le danger de lavage de la pellicule d'insecticide sur la surface des organes de la plante. Aussi, on attend que la pénétration, ainsi que le degré d'utilisation des insecticides, soient plus grands que dans le cas des insecticides qui sont administrés par pulvérisation ou arrosage.

Les insecticides à fonctions systémiques présentent une importance particulière dans la protection des forêts, parce que les espèces ligneuses sont attaquées par des insectes qui se localisent soit dans le bois, soit entre

l'écorce et le bois. La lutte contre ces insectes nuisibles est donc impossible avec des insecticides sans fonction systémique.

Parmi les espèces ligneuses cultivées dans la République Populaire Roumaine, ce sont les peupliers euraméricains ainsi que les peupliers indigènes qui souffrent le plus des attaques d'insectes se localisant dans les bois. La Saperda populnea L., Paranthrenae tabaniformis Rott., Crypthorrinchus lapathi L., etc. appartiennent aux insectes nuisibles les plus répandus. Les larves de ces insectes se développent dans le bois de peuplier. En détruisant les exemplaires attaqués elles compromettent les jeunes peuplements.

L'application des insecticides systémiques dans ce domaine est devenue plus qu'une nécessité. Mais, vu la difficulté de suivre la pénétration de l'insecticide dans la plante, les méthodes d'administration n'ont pas été explorées scientifiquement, ce qui a empêché leur application sur une grande échelle.

La possibilité de marquer les insecticides systémiques avec des isotopes radioactifs a ouvert une nouvelle perspective aux recherches dans ce domaine. Vu l'importance de la lutte contre les insectes nuisibles du peuplier et afin d'assurer un état phytosanitaire optimum des cultures de peupliers dans notre pays, on a entrepris une série de recherches pour étudier en détails l'efficacité des insecticides systémiques par rapport à la méthode d'administration aux insectes nuisibles, aux espèces et aux clônes de peupliers et aux facteurs écologiques.

Les premières recherches qui font l'objet du présent mémoire ont comme but l'éclaircissement des processus d'absorption et diffusion du dipterex marqué dans les plants de Populus x euramericana Dode Guinier, cv. «robusta».

2. MÉTHODES DE RECHERCHES

2.1. La conception de base des recherches entreprises par le Laboratoire d'isotopes radioactifs de notre Institut, a été déterminée par le but précis des recherches en espèce, et par l'analyse des méthodes de travail utilisées jusqu'à présent.

Le degré de pénétration des insecticides systémiques dans le flux de la sève des espèces ligneuses a été établi d'après la mortalité des insectes qui attaquent les espèces respectives. Comme méthode d'administration de ces insecticides, on a eu recours soit à la pulvérisation fine sur les feuilles et les branches, soit à l'introduction de l'insecticide dans le sol. Parfois on a essayé le bandage de la tige avec de la ouate imbibée d'une solution d'insecticide.

Dans les premières expériences faites dans notre pays en vue de déterminer l'efficacité des insecticides systémiques, on a choisi comme méthode d'administration la pulvérisation de l'insecticide sur les feuilles, considérant qu'une proportion importante s'intègre dans le courant descendant de la sève.

JEPPSON a recours à une méthode moins pratique mais plus efficace. Il a appliqué des bandages de ouate imbibée avec metasystox d'une concen-

tration de 2,5% et 5%, autour de la tige des exemplaires de mélèze attaqués par Taeniothrips laricivorus Krat [1].

Ces méthodes ont permis la détermination de l'efficacité de l'insecticide basée sur les observations faites sur la mortalité des insectes et les différences de croissance enregistrées par les exemplaires des variantes traitées avec des insecticides systémiques et celles non traitées (contrôle).

Les progrès réalisés dans le domaine du marquage des molécules de divers composés chimiques, ainsi que la présence du phosphore, du soufre et du carbone dans la molécule des insecticides systémiques, ont permis aisément le marquage des insecticides organophosphoriques.

ANDREEV [2], CASIDA [3] et HOPKINS [4] ont utilisé dans les recherches effectuées, des insecticides systémiques marqués par une synthèse chimique. Andreev a poursuivi l'absorption du mercaptophos marqué par les plantes et a établi les concentrations létales et la localisation de l'insecticide dans les plantes.

En général, les recherches dans ce domaine ont pour objet les cycles métaboliques des insecticides systémiques dans les plantes et dans l'organisme des insectes, poursuivis à l'aide des composés marqués aux isotopes radioactifs et mis en évidence par chromatographie radiochimique [2, 3, 4, 5].

2.2. Les recherches entreprises par notre Institut ont le but d'éclaircir tout d'abord la quantité d'insecticides systémiques qui s'accumule dans les plants de Populus x euramericana, cv. «robusta». On a utilisé du dipterex marqué au réacteur nucléaire de l'Institut de physique atomique de l'Académie de la République Populaire Roumaine.

On a obtenu le dipterex radioactif par marquage du phosphore, avec une activité spécifique initiale de 1 mc/g. On a effectué les expériences, soit seulement avec l'insecticide marqué, soit avec l'insecticide marqué auquel on a ajouté l'insecticide stable, par rapport aux concentrations établies. On a travaillé d'habitude avec des concentrations de 1-2% dans une solution aqueuse.

On a administré les solutions de dipterex dans la première dilution (10 μc/ml) dans le sol à la racine des plants de peuplier, on a poussé une partie dans de vases de végétation, et une autre partie dans la pépinière. Pour la mobilisation de l'insecticide par les plantes, on a assuré une percolation intense du sol avec de l'eau.

On a observé le degré d'absorption de l'insecticide à l'aide de l'intensimètre «Luci» portable.

Au moment où les mesures radiométriques indiquaient une activité maximale à la cime des plants, on a enlevé celle-ci pour préparer des échantillons afin de procéder à des mesures exactes à l'aide du dispositif de comptage BSP, avec compteur G-M, type MST-17, à la fenêtre mica de 5 mg/cm^2.

L'activité des échantillons prélevés en diverses parties de la plante a été déterminée à l'aide d'échantillons calcinés. Les résultats des mesures permettent de déterminer avec une précision suffisante la diffusion du dipterex dans diverses parties de la plante, la localisation et la quantité de l'insecticide absorbé par rapport à la quantité administrée.

3. RÉSULTATS DES RECHERCHES

Les recherches ont été effectuées en deux étapes, à savoir, une étape au laboratoire et une étape sur le terrain. Les résultats obtenus diffèrent évidemment surtout en ce qui concerne la quantité de dipterex absorbé du sol par les plants de peuplier.

3.1. Les expériences de laboratoire ont été effectuées avec des plants âgés d'une année, plantés dans de vases de végétation Mitscherlich avec une capacité de 20 l. Dans les vases on a utilisé un sol alluvionaire à texture sablonneuse, riche en humus.

Les solutions de dipterex marqué ont été administrées à la racine des plants, en concentration de 1,5%. L'activité spécifique des solutions a été $R_0 = 5340$ cpm/ml. L'activité totale sur la plante a été de $5,34 \cdot 10^6$ cpm.

On a vérifié périodiquement l'accroissement de l'activité dans les feuilles, et 30 jours après l'administration de la solution de dipterex marqué on a prélevé des échantillons de bois et de feuilles soumis ultérieurement aux mesures radiométriques, à l'état calciné.

Les déterminations faites montrent que le dipterex marqué a été absorbé par les plants et qu'il est distribué différemment dans le bois et dans les feuilles, sur les secteurs de 20 cm mesurés sur la tige.

L'activité totale (cpm) est plus grande dans les secteurs dans lesquels la masse ligneuse ou la masse de feuilles est plus grande et en général décroît vers la tête des plants (tableau I).

En échange, l'activité spécifique exprimée en cpm/g (cendre), montre une accumulation plus intense de l'insecticide dans les racines et dans la moitié supérieure de la tige, surtout aux 3/5 de la hauteur. Dans les feuilles l'activité spécifique en cpm/g (cendre) est distinctement plus grande que dans le bois et accumule une quantité plus grande d'insecticide aussi aux 3/5 de la hauteur de la plante et vers le bout de la plante. On remarque

TABLEAU I

LA VARIATION DE L'ACTIVITÉ TOTALE EN cpm DANS LES DIVERSES PARTIES DES PLANTS DE PEUPLIER (POPULUS ROBUSTA) TRAITÉS AU DIPTEREX MARQUÉ (EXPÉRIENCE DE LABORATOIRE)

Partie de la plante	La hauteur à partir du sol (cm)				
	0-20	20-40	40-60	60-80	80-100
Feuilles	-	723	1518	854	652
Bois	254	184	274	137	57
Bouttures	1990				
Racines	580				

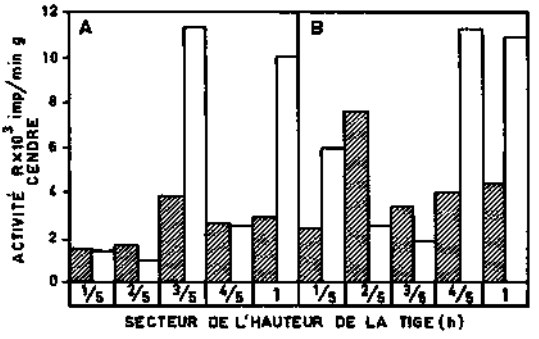

Figure 1

La variation de l'activité rapportée à l'unité de masse calcinée, en cpm/g (cendre), dans les diverses parties des plants de peuplier traités avec dipterex marqué, sur divers secteurs de la hauteur de la tige.

▨ - Bois tige; □ - Feuilles.

A. Expérience de laboratoire; B. Expérience de terrain.

une tendance de polarité du dipterex marqué vers les extrémités de la plante, avec la présence d'un troisième maximum aux 3/5 de la hauteur des plants (fig. 1).

Dans le bois de la tige, l'activité varie entre 1500-3800 cpm/g (cendre) et dans les feuilles entre 2400-7600 cpm/g (cendre). Par conséquent, l'accumulation d'insecticide dans les feuilles s'avère être deux fois plus grande que dans le bois. Les différences sont encore plus grandes si l'on prend en considération les valeurs de l'activité rapportée à la substance sèche, parce que le contenu en cendre du bois varie entre 3-4%, et celui des feuilles entre 8-9%.

Il résulte des expériences de laboratoire que l'absorption du dipterex par les plants de P. robusta représente 1,34% de la quantité administrée; la mobilisation de l'insecticide en proportion réduite dans ces expériences est due, d'une part, à la percolation intense du sol et à la présence de l'insecticide dans l'eau d'arrosage et, d'autre part, aux conditions écologiques du laboratoire, qui ne permettent pas une activité physiologique intense des plantes.

De la dernière analyse il résulte que l'accumulation d'insecticide dans le bois est de 0,172 mg/g, et dans les feuilles de 0,373 mg/g, substance verte.

3.2. Les expériences sur le terrain montrent qu'en général, l'absorption et la diffusion du dipterex dans les plants de P. robusta se produisent de la même manière que dans l'expérience de laboratoire, à la différence que ces processus sont beaucoup plus intenses.

L'expérience s'est déroulée avec la même hypothèse et la méthode de travail est semblable à celle utilisée dans les expériences de laboratoire.

La solution active de dipterex dans la première dilution (d_1) a eu une activité $R_{01} = 22\,200$ cpm/ml. A chaque exemplaire de peuplier on a administré $R_{02} = 666$ cpm/ml. L'activité totale de la solution administrée à un exemplaire de peuplier a été de $6{,}66 \cdot 10^5$ cpm, donc du même ordre de grandeur que dans le cas de l'expérience de laboratoire.

Au dipterex marqué on a ajouté aussi le dipterex stable de telle manière que la concentration de la solution d'insecticide fut de 1%. L'activité du dipterex a été de $6,6 \cdot 10^4$ cpm/g (dipterex).

On a observé le progrès de l'absorption de l'insecticide et lors d'une accumulation d'activité facilement mesurable on a prélevé les échantillons pour les mesures radiométriques.

L'activité de la substance, à l'état calciné, montre le même phénomène de polarité dans l'accumulation du dipterex marqué que dans le cas de l'expérience de laboratoire. Dans la tige l'activité maximum apparaît aux 3/5 de la hauteur et est de 11 340 cpm/g (cendre), dans les rameaux aux 2/5 et au sommet avec des valeurs de 28 400 et de 23 100 cpm/g (cendre), respectivement, dans le pétiole des feuilles aux 4/5 et 1/5 avec des valeurs de 13 200 et de 7400 cpm/g (cendre), respectivement, et dans les feuilles aux 2/5 et au bout avec des valeurs de 11 250 et 5870 cpm/g (cendre), respectivement (tableau II).

TABLEAU II

LA VARIATION DE L'ACTIVITÉ EN cpm/g (CENDRE) DANS LES DIVERSES PARTIES DES PLANTS DE PEUPLIER TRAITÉS AU DIPTEREX MARQUÉ (EXPÉRIENCE DE TERRAIN)

La hauteur à partir du sol (cm)	Parties de la plante			
	Bois	Branches	Pétiole feuilles	Feuilles
0-50	10 000	-	7400	10 850
50-100	2500	28400	4150	11250
100-150	11340	2500	4160	1830
150-200	960	1430	13200	2490
200-250	1400	23100	1700	5870

En comparaison avec les mêmes valeurs trouvées dans l'expérience de laboratoire, les plants de peupliers élevés dans des conditions de terrain en pleine saison de végétation mobilisent l'insecticide dans une proportion 3-4 fois plus grande sur l'unité de masse.

Contrairement à l'expérience précédente, on constate une augmentation de la radioactivité dans le bois de la tige et les rameaux qui dépasse les valeurs enregistrées dans les feuilles, phénomène inverse à celui enregistré auprès des plants en pleine croissance dans des conditions de laboratoire. Il en résulte que les insecticides systémiques diffusent aussi dans de divers organes des plantes, selon la même loi que la sève élaborée et en concordance parfaite avec les composés de biosynthèse. Dans la première phase d'accroissement, au début de la saison de végétation, le dipterex s'accumule plus fortement dans les feuilles, et dans la seconde partie de la saison

de végétation dans le bois. Ce phénomène est d'un grand intérêt d'ordre pratique.

L'accumulation de l'insecticide dans les plants vigoureux de P. robusta de deux ans se produit à raison de 10,8% dans le bois et de 8,6% dans les feuilles par rapport à la quantité totale de dipterex administrée à un exemplaire.

En unité de masse on peut dire que dans le bois s'est accumulée une quantité de dipterex de 1,1 g, et dans les feuilles de 0,9 g, donc, au total, approximativement 2 g des 10 g administrées à une plante.

L'absorption d'environ 20% du dipterex par plants de P. robusta au mois d'août de l'année 1962 montre que l'utilisation des insecticides systémiques administrés dans le sol peut donner des résultats remarquables dans la lutte contre les insectes. Nous considérons cependant que, dans ce cas, il est utile d'augmenter la concentration des solutions jusqu'à 5%, fait relevé d'ailleurs aussi par JEPPSON [1] dans un autre genre d'expériences.

On a observé qu'à la suite de l'infestation artificielle intense des plants de peuplier avec Saperda populnea, les plants ont été faiblement attaqués et les larves dans le bois sont mortes. La quantité d'insecticide accumulée dans le bois a été de 1,81 mg/g et dans les feuilles de 2,18 mg/g de substance verte, presque plus grande donc que dans le cas de l'expérience de laboratoire. On a observé également une faible absorption du dipterex chez les plants auxquels on a administré l'insecticide par voie extraradiculaire, grâce au lent processus d'absorption par les feuilles ainsi qu'au lavage de celles-ci par les eaux de pluies.

Il résulte donc que les processus d'absorption et diffusion des insecticides systémiques sont déterminés dans une mesure appréciable par les conditions météorologiques, par l'intensité des processus physiologiques et par la vigueur des cultures de peupliers.

4. CONCLUSIONS

4.1. L'utilisation des traceurs radioactifs permet l'étude de l'absorption et de la circulation des insecticides organo-phosphoriques aux fonctions systémiques dans le système sol-plante.

4.2. Les plants de P. robusta absorbent, dans les limites convenables, le dipterex administré dans le sol, et la localisation de l'insecticide dans les organes divers peut assurer une immunité de la plante envers les divers insectes nuisibles.

4.3. Dans les conditions de laboratoire et dans la première phase de croissance, l'absorption de l'insecticide est plus faible, tandis que dans les conditions de terrain en cultures intensives, environ 20% de la quantité administrée sont absorbés.

4.4. Dans la première partie de la saison de végétation, l'accumulation de l'insecticide est plus intense dans les feuilles que dans le bois, tandis que dans la seconde partie de la saison, l'accumulation de celui-ci dans le bois augmente d'une manière considérable (dans une plus grande proportion).

4.5. On remarque un phénomène distinct de polarité dans la diffusion de l'insecticide dans les plants. Toutefois, il apparaît une zone d'accumulation intense de l'insecticide dans la partie inférieure de la couronne des plants, tant dans les feuilles que dans le bois, plus prononcée dans le tiers moyen de la tige des plants.

4.6. Les aspects concernant la stabilité du dipterex et des autres insecticides systémiques constituent l'étape nouvelle des recherches entreprises, et l'établissement des concentrations optima à administrer dans le sol est un problème qu'on est en train d'étudier. Bien que les dates obtenues jusqu'à présent tendent de montrer que certains insecticides systémiques se décomposent après 19-24 jours [2], nous considérons que ce problème doit encore être examiné, surtout dans l'hypothèse de l'administration des solutions de l'insecticide dans le sol. Le phénomène doit être relié surtout au pH de la solution du sol et de la phase liquide contenue dans les plantes.

4.7. Les préoccupations concernant l'utilisation sur une large échelle des insecticides systémiques dans la sylviculture pratique sont en plein développement, et nous considérons que la protection des forêts offre le plus important domaine d'application de ces substances.

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DISCUSSION

J.E. CASIDA: Dipterex is known to be a fairly good systemic in certain trees. It is also somewhat unstable biologically under these circumstances - the period of stability can be estimated at 2 to 10 days, on the basis of bioassays not restricted by localization. I am wondering if this might have some effect on the interpretation of your results. In other words, do you know for sure that you are analysing the active insecticide? Was any attempt made to ascertain that the radioactivity measured was due to Dipterex per se and not to non-insecticide metabolites?

C.H. SCHMIDT: Yes, I also wanted to ask this question.

I. CATRINA: As regards the period of stability of Dipterex, the research carried out in my country has indicated a rather longer period, i.e. about 30 days. Research work on the problem is still going on. You are certainly quite right, Dr. Casida, in saying that Dipterex decomposes, especially when it is applied topically to plants. The main cause of this phenomenon is the ultra-violet ray effect. In the case of injection of Dipterex into the soil, however, this particular phenomenon is eliminated.

There is no doubt that the radioactivity we measured was due to the Dipterex itself. The biological test used showed it to be Dipterex and not non-insecticide metabolites. The absorption rate was too rapid, the activity appeared too soon for it to have been otherwise. The dead larvae in the wood and the mortality among larvae fed on the leaves suffice to show the presence of Dipterex itself.

G. F. BURNETT (Chairman): It seems to me you are using very large amounts of Dipterex, 10 g to a very small tree. Your 20% utilization is high, and I assume in practice you will use very much smaller quantities. Have you considered that in such a case the relative distribution of Dipterex in wood and leaves may be different and fall below the effective concentration in the wood? Are you pursuing any studies along this line to see whether with, say 1 g of Dipterex you will get the same type of distribution throughout the tree?

I. CATRINA: The 10-g dose which we used for a two-year-old sapling of Populus robusta was not too large because the insecticide was administered into the soil. We have not studied dosage from the practical point of view. That is for the future.

T. SAITO: Do you do purification of chemical assays of used radioactive Dipterex?

I. CATRINA: After labelling we do no purification or chemical assays of Dipterex because we receive the radioactive insecticide from a radiochemical laboratory as pure labelled Dipterex, and its purity is guaranteed.

I might add that paper-chromatographic studies made at the Institute of Atomic Physics of the Romanian Academy of Sciences aim at establishing the quality of labelled insecticides as we use them in our experiments.

RADIOTRACER APPROACHES TO CARBAMATE INSECTICIDE TOXICOLOGY

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Abstract — Résumé — Аннотация — Resumen

RADIOTRACER APPROACHES TO CARBAMATE INSECTICIDE TOXICOLOGY. Methylcarbamates constitute one of the major groups of insecticides. Many unresolved problems in their toxicology may be readily approached with radiotracer studies. Dimethylcarbamates have been prepared with carbonyl-C^{14}-labelling and methylcarbamates with methyl-, carbonyl- and ring-labelling utilizing carbon-14. The pharmacological action of these compounds presumably results from acetylcholinesterase inhibition and may involve carbamylation. Reaction of carbonyl- or methyl-labelled carbamates with purified cholinesterase or other esterases would allow a critical examination of this carbamylation reaction and the ease of spontaneous and induced reactivation or decarbamylation. The physiological significance of cholinesterase inhibition might be examined by administering acetate-C^{14} and analysis for radiolabelled acetylcholine accumulation in nervous tissue, or by utilizing acetyl-C^{14}-choline as the substrate for in vitro determination of the degree of cholinesterase inhibition in tissues of poisoned animals with minimal dilution of the inhibitors and enzymes during analysis. Some progress has been made with radiolabelled materials in investigating the metabolism of carbamate insecticides. Sevin (1-naphthyl methylcarbamate) has been most extensively studied along with its potential hydrolysis products. The assumption that the metabolism of Sevin involves an initial hydrolysis and then further decomposition of the fragments was not supported by carbon-14 studies. The major detoxification mechanism in mammals, and probably also in insects, results from initial oxidative attack on the carbamate by the microsomes in the presence of reduced nicotinamide-adenine dinucleotide phosphate. Sevin is rapidly metabolized in mammals, but the fate of certain of the fragments has not been resolved. Some of the metabolites appear in the milk of lactating animals. One step in the metabolism appears to be formation of the N-methylol derivative. Preliminary studies on the metabolism of radiolabelled Dimetilan (2-dimethylcarbamyl-3-methylpyrazolyl-(5)-dimethylcarbamate) and a related compound in cockroaches also indicate that oxidative attack forms N-methyl N-methylol derivatives. Much remains to be done on the relationship of these detoxification reactions to the resistance mechanism, the action of synergists, the selective toxicity in this group of insecticides, and the nature and significance of residues. Metabolism of Sevin following injection into plants is probably also oxidative rather than hydrolytic, but the nature of the products and the enzymatic mechanism have not yet been established.

EMPLOI DES RADIOINDICATEURS POUR L'ÉTUDE DE LA TOXICOLOGIE DES INSECTICIDES A BASE DE CARBAMATES. Les méthylcarbamates constituent l'un des principaux groupes d'insecticides. Les radioindicateurs permettent d'étudier facilement de nombreux problèmes que pose leur toxicologie. On a préparé des dimethylcarbamates en marquant le groupe carbonyle par ^{14}C, et des méthylcarbamates en marquant par ^{14}C les groupes méthyle et carbonyle et le noyau. L'action pharmacologique de ces composés résulte vraisemblablement de l'inhibition de l'acétylcholinestérase et peut impliquer une carbamylation. La réaction, avec de la cholinestérase ou d'autres estérases purifiées, des carbamates dont on a marqué les groupe carbonyle et méthyle permettrait de faire une étude critique de cette carbamylation et de la facilité avec laquelle se produit la réactivation ou décarbamylation, spontanée ou induite. L'importance physiologique de l'inhibition de la cholinestérase peut être étudiée en administrant de l'acétate marqué par ^{14}C et en analysant l'accumulation de l'acétylcholine marquée dans les tissus nerveux, ou bien en utilisant de l'acétyl-^{14}C-choline comme substrat pour déterminer in vitro le degré d'inhibition de la cholinestérase dans les tissus d'animaux traités, avec une dilution minimum des inhibiteurs et des enzymes pendant l'analyse. Les produits marqués ont permis certains progrès dans l'étude du métabolisme des insecticides à base de carbamates. On a étudié de manière très approfondie le Sevin (1-naphtyl méthylcarbamate), en même temps que ses produits d'hydrolyse éventuels. L'hypothèse selon laquelle le métabolisme du Sevin implique une hydrolyse initiale, puis une nouvelle décomposition des produits d'hydrolyse, n'a pas été confirmée par les études faites avec le carbone-14. Chez les mammifères et probablement aussi chez les insectes, la détoxification résulte principalement d'une oxydation initiale du carbamate par les microsomes en présence de phosphate de nicotinamide adénide dinucléotide réduit. La

métabolisation du Sevin est rapide chez les mammifères, mais on n'a pas encore pu déterminer le sort de certains des fragments. Quelques métabolites apparaissent dans le lait des animaux en lactation. L'une des étapes du métabolisme semble être la formation d'un dérivé de N-méthylol. Des études préliminaires sur le métabolisme du Dimétilan radiomarqué (diméthylcarbamate de 5-[3-méthyl-2 diméthylcarbamyl]-pyrazoline) et d'un composé voisin chez les blattes a montré également que l'oxydation forme des dérivés de N-méthyl-N-méthylol. Il reste encore beaucoup à faire pour déterminer le rapport qui existe entre ces réactions de détoxification et la résistance, l'action des agents synergétiques et la toxicité sélective dans ce groupe d'insecticides, ainsi que pour déterminer la nature et l'importance des résidus. Il est également probable que le métabolisme du Sevin, à la suite d'injection dans des plantes, suit une voie oxydante plutôt qu'une voie hydrolytique, mais la nature des produits et le mécanisme enzymatique n'ont pas encore été déterminés.

ПРИМЕНЕНИЕ РАДИОАКТИВНЫХ ИНДИКАТОРОВ ДЛЯ ИЗУЧЕНИЯ ТОКСИКОЛОГИИ КАРБАМАТНЫХ ИНСЕКТИЦИДОВ. Метилкарбаматы являются одной из главных групп инсектицидов. Многие неразрешенные проблемы токсикологии этих веществ могут быть легко изучены при помощи радиоактивных индикаторов. Были изготовлены N, N-диметилкарбаматы, карбонильная группа которых метилась C^{14}, а также N-метилкарбаматы, метильная, карбонильная группа и кольца которых были мечены C^{14}. Фармакологическое действие этих сложных соединений, вероятно, связано с торможением ацетилхолинэстеразы и может включать карбамилацию. Мечение карбонильной или метильной группы карбаматов дает возможность в их реакциях с очищенной холинэстеразой или с другими эстеразами критически изучить реакцию карбамилации, а также легкость самопроизвольной или вызванной реактивации или декарбамилации. Физиологическое значение торможения холинэстеразы может быть изучено при помощи введения меченного C^{14} ацетата и анализа меченого ацетилхолина, накопившегося в нервных тканях, или же путем использования ацетил-C^{14}-холина в качестве субстрата для определения in vitro степени торможения холинэстеразы в тканях отравленных животных при минимальном растворении во время анализа ингибиторов и ферментов. Некоторые успехи были достигнуты при помощи меченных радиоизотопами веществ в исследовании метаболизма карбаматных инсектицидов. Севин (1-naphtyl N-methylcarbamate) был наиболее тщательно изучен параллельно с возможными продуктами его гидролиза. Исследования с помощью углерода-14 не подтвердили гипотезы о том, что при метаболизме севина происходит первоначальный гидролиз, а затем дальнейшее разложение продуктов гидролиза. Основной механизм обезвреживания инсектицида в организме млекопитающих и, вероятно, насекомых заключается в первоначальном окисляющем воздействии микросом на карбаматы в присутствии восстановленного никотинамид-аденинди-нуклеотид фосфата. Севин быстро подвергается распаду у млекопитающих, но судьба некоторых продуктов распада еще не выяснена. Некоторые из продуктов метаболизма могут быть обнаружены в молоке животных. Одной из ступеней метаболизма, по-видимому, является образование производного N-метилола. Предварительные исследования метаболизма меченного радиоактивными веществами диметилана (2-диметилкарбамид-3-метилпиразолил-(5)-диметилкарбамат) и метаболизма подобного соединения у тараканов указывает также на то, что при окислении образуются производные N-метила и N-метилола. Многое остается сделать для выяснения связи этих реакций обезвреживания с механизмом сопротивляемости, действием синергистов, селективной токсичностью этой группы инсектицидов, а также с природой и значением остатков инсектицида. Метаболизм севина, введенного в растения, носит, вероятно, также скорее окислительный, а не гидролитический характер, но характер продуктов и ферментативные механизмы еще не установлены.

ESTUDIO CON RADIOINDICADORES DE LA TOXICOLOGÍA DE LOS INSECTICIDAS A BASE DE CARBAMATOS. Los metilcarbamatos constituyen uno de los principales grupos de insecticidas. Muchos de los problemas que su toxicología plantea se pueden estudiar fácilmente mediante radioindicadores. Se han preparado dimetilcarbamatos marcados con ^{14}C en el grupo carbonilo, y metilcarbamatos marcados con ^{14}C en los grupos metilo, carbonilo y, en el caso de derivados cíclicos, en el anillo. La acción farmacológica de estos compuestos se debe probablemente a la inhibición de la acetilcolinesterasa y es posible que implique una carbamilación. La reacción con colinesterasa purificada u otras esterasas de los carbamatos marcados en los grupos carbonilo o metilo permitiría un examen crítico de esta reacción de carbamilación y de la tendencia a la reactivación o descarbamilación espontánea e inducida. La importancia fisiológica de la inhibición de la colinesterasa se podría estudiar administrando acetato-^{14}C y analizando la acumulación de acetilcolina marcada en el tejido nervioso, o bien utilizando acetil-^{14}C-colina como substrato para la determinación in vitro del grado de inhibición de la colinesterasa en tejidos de animales envenenados, con una dilución mínima de los inhibidores y las enzimas durante el análisis. El empleo de sustancias marcadas ha permitido realizar

algunos progresos en la investigación del metabolismo de los insecticidas a base de carbamato. Se ha estudiado principalmente el Sevin (metilcarbamato de 1-naftilo) y sus posibles productos de hidrólisis. Los estudios con ^{14}C no corroboraron la hipótesis de que el metabolismo del Sevin implica una hidrólisis inicial seguida de una mayor descomposición de los fragmentos. La principal desintoxicación en los mamíferos, y probablemente también en los insectos, es el resultado de un ataque oxidante inicial del carbamato por los microsomas en presencia de fosfato de nicotinamida-adenina-dinucleótido reducido. Los mamíferos metabolizan rápidamente el Sevin, pero no se ha averiguado todavía la evolución ulterior de algunos de sus fragmentos. Algunos metabolitos aparecen en la leche de animales lactantes. Al parecer, una de las etapas metabólicas es la formación de un derivado N-metilol. Estudios preliminares, efectuados en la cucaracha, del metabolismo del Dimetilan radiomarcado (dimetilcarbamato de 5-[3-metil-2-dimetilcarbamil]- pirazolina) y un compuesto comparable indican también que el ataque oxidante forma derivados N-metilo y N-metilol. Faltan todavía muchos datos sobre la relación entre esas reacciones de desintoxicación y el mecanismo de resistencia, la acción de las sustancias sinérgicas, la toxicidad selectiva de este grupo de insecticidas, y la naturaleza y el significado de los residuos. Es probable también que el metabolismo del Sevin inyectado en las plantas sea oxidativo más que hidrolítico, pero no se ha establecido todavía la naturaleza de los productos y el mecanismo enzimático.

INTRODUCTION

Insecticidal carbamic acid esters have been extensively investigated during the past decade. Two dimethylcarbamates, Isolan (1-isopropyl-3-3-methylpyrazolyl-(5)-dimethylcarbamate, compound VIII of Fig. 1) and Dimetilan (2-dimethylcarbamyl-3-methylpyrazolyl-(5)-dimethylcarbamate, compound VII of Fig. 1), are in limited use, and one monomethylcarbamate, Sevin (1-naphthyl methylcarbamate, compound I of Fig. 1), is extensively utilized for pest control. Several other methylcarbamates have been thoroughly field-tested for insecticidal activity, and their residual persistence and toxicology are now being evaluated. Recent reviews have considered the development, mode of action and present status of these carbamates [1-8].

Investigations of certain aspects of the mode of action of the carbamate insecticides, particularly those dealing with their detoxification mechanisms, are greatly facilitated by radiotracer approaches. Although few such studies have been reported, many are now in progress. An attempt will be made to review the information obtained to date from such studies, and to indicate where tagged molecules might further assist in clarifying unresolved or incompletely elucidated facets of the mode of action of the carbamate insecticides.

1. RADIOSYNTHESIS

The insecticidal carbamates of current interest are all methyl- or dimethylcarbamates of substituted phenols or heterocyclic enols. Two routes of radiosynthesis are obvious for the dimethylcarbamates and three for the methylcarbamates:

1. Via the carbamyl chloride

$$COCl_2 + 2\,(CH_3)(R)NH \rightarrow (CH_3)(R)NC(O)Cl + (CH_3)(R)NH \cdot HCl$$

$$\downarrow \text{ ROH or RONa}$$

$$(CH_3)(R)NC(O)OR + HCl \text{ or } NaCl$$

2. Via the chloroformate

$$COCl_2 + ROH \xrightarrow{-HCl} ClC(O)OR \xrightarrow{2(CH_3)(R)NH} (CH_3)(R)NC(O)OR + (CH_3)(R)NH \cdot HCl$$

2. Via the isocyanate

$$CH_3NCO + ROH \rightarrow (CH_3)(H)NC(O)OR$$

The first route has been used by Dr. D. P. Ryskiewich (Geigy Chemical Corporation, Ardsley, New York) to prepare carbonyl-C^{14}-dimethylcarbamyl chloride in almost quantitative yields. A total of 3 mM of phosgene-C^{14} in 3.6 ml dry benzene was transferred to a 25-ml reaction flask equipped with a condenser, drying tube and magnetic pellet in an ice bath. A total of 6 mM of dimethylamine in 6.6 ml dry benzene was added with stirring in 1-ml portions at 10-min intervals. The resulting slurry was then stirred at room temperature for one hour. The benzene solution of carbonyl-C^{14}-dimethylcarbamyl chloride was separated from the insoluble dimethylamine hydrochloride by filtration and used directly by Dr. Ryskiewich in the preparation of the dimethylcarbamates, Dimetilan (VII) and Isolan (VIII). By substituting anhydrous methylamine for the dimethylamine, it should be possible to prepare carbonyl-C^{14}-methylcarbamyl chloride under similar reaction conditions. This reaction could also be used to prepare the methyl-C^{14}- or methyl-H^3-labelled methylcarbamyl chlorides by providing a means of regenerating the amines from the hydrochloride for completion of the reaction of methyl-C^{14}-amine or methyl-H^3-amine with phosgene. Reaction conditions suitable for the final coupling to form the carbamate ester have been reported [9–15].

The second route has been used for radiolabelling carbamates of pharmacological interest but not for the methylcarbamate insecticides. For example, in the preparation of 1-ethynylcyclohexyl carbamate-carbonyl-C^{14}, the reactions were carried out in collidine at -10°C without isolation of the intermediate chloroformate [16].

Sevin (I) has been prepared by the reaction of 1-naphthol-1-C^{14} with methyl isocyanate [17, 18]. Detailed conditions for this reaction conducted in a solution of 2% pyridine in dry benzene at 80°C for 5 h have been reported (17). The product after recrystallization from hot p-xylene was recovered in 82% yield and characterized by melting point and isotopic dilution analysis. Methyl isocyanate-C^{14} has also been prepared and reacted to yield labelled methylcarbamate insecticides [19] using the following reaction sequence and conditions adapted from reported procedures for non-labelled materials [20, 21]:

$$CH_3C(O)Cl + NaN_3 \xrightarrow[140°C,\ 2\ h]{\text{benzene}} CH_3NCO + NaCl + N_2$$

$$CH_3NCO \xrightarrow{100°C,\ 2\ h,\ ROH,\ (C_2H_5)_3N} CH_3NHC(O)OR$$

Acetyl chloride, labelled with carbon-14 in either the methyl or carbonyl position, and sodium azide were reacted to yield methyl isocyanate-C^{14}, which was then reacted with the appropriate phenol. A two-compartment reaction tube with a break-seal was utilized. Yields on a 0.5-mM scale were routinely 40-70%. The methylcarbamates were isolated by chromatography on Florisil and crystallization. Their purity and structure were confirmed by infra-red spectra, co-chromatography on Florisil columns and silica-gel thin-layer chromatograms, and by their melting points and mixed melting points. Acetyl-1-C^{14} chloride or bromide yielded carbonyl-C^{14}-methylcarbamates, and acetyl-2-C^{14} chloride or bromide yielded the methyl-C^{14}-carbamates. This method has been modified (Dr. Roy Everett, Chemagro Corporation, Kansas City, Missouri) by introducing the acetyl chloride into the reaction tube with a vacuum manifold rather than as a benzene solution to minimize water pick-up, and by completing the first reaction at the higher temperature before introducing the substituted phenol in the case of thermally unstable phenols. Further useful modifications of this procedure include preparation of the methyl isocyanate-C^{14} followed by introduction of the substituted phenol into the other compartment of the reaction tube without triethylamine, and reacting the phenol and isocyanate for either 2 h at 100°C or several days at room temperature [22]. Sevin-carbonyl-C^{14}, as prepared via methyl isocyanate-C^{14}, is available from Volk Radiochemical Co. (Skokie, Illinois). This break-seal tube and reaction sequence should also be appropriate for preparing methyl-H^3-carbamates from acetyl-H^3-chloride or acetic-H^3-anhydride.

The carbon-14-labelled carbamate insecticides currently under investigation are indicated in Fig. 1. The available evidence on the metabolism of these insecticides has emphasized the importance of working with multiple sites of labelling. The toxicological studies discussed later have already reached the point where it would be advantageous to utilize carbonyl-C^{14} and methyl-H^3 labels on the same methylcarbamate, or carbonyl-C^{14} and tritium labelling of the enolic moiety within the same molecule. The synthesis of certain of these insecticides with carbon-14 at selected sites in the rings or in a ring substituent should also be readily possible with available routes.

2. METABOLISM OF METHYLCARBAMATES

The first reported study utilizing radiolabelled carbamate insecticides dealt with a comparison of the metabolism of naphtyl-1-C^{14}-Sevin and 1-naphthol-1-C^{14} in three insect species [18]. The metabolites were separated by a paper chromatographic system, with glutaronitrile as the stationary phase and isopropyl ether saturated with glutaronitrile as the ascending mobile phase. With a normal susceptible (S) strain of house-fly, Musca domestica L., Sevin penetrated rapidly either alone or with the synergist, sesamex [2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane], but sesamex interfered with the process or processes by which the absorbed Sevin was metabolized and excreted. Sevin was rapidly changed to a very polar substance that appeared both in the tissues and the excreta. With sesamex there was a much greater amount of unchanged Sevin in the body and much

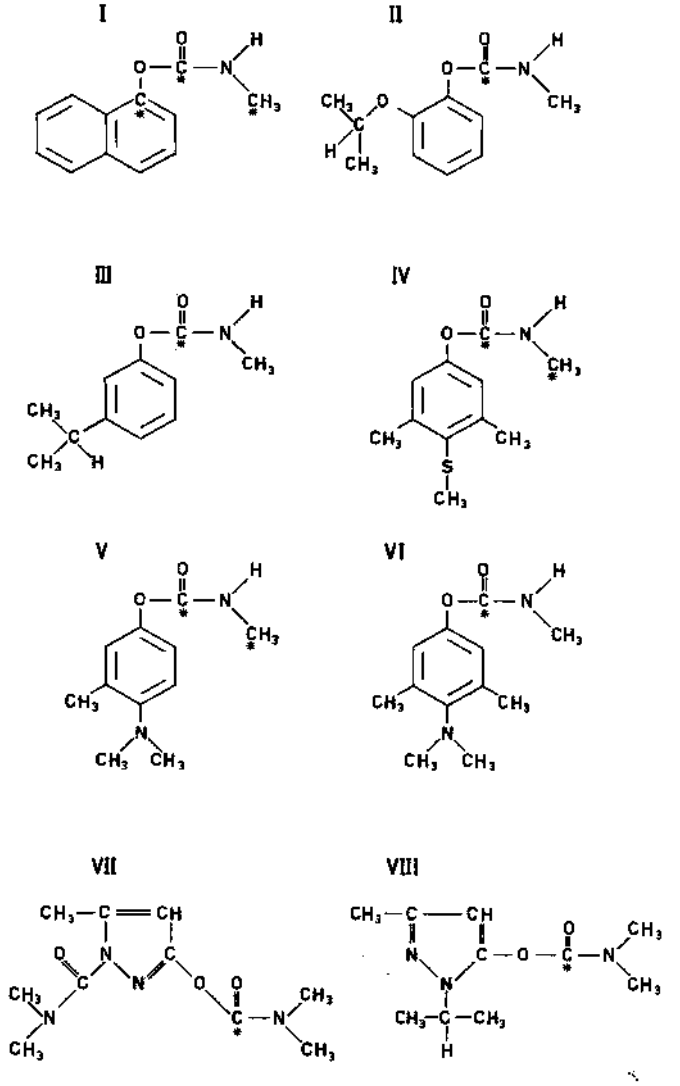

Fig. 1

Sites of C^{14}-labelling (marked *) in carbamate insecticides currently being used in radiotracer investigations

I. Sevin: 1-naphthyl methylcarbamate
II. Bayer 39007: o-isopropoxyphenyl methylcarbamate
III. Hercules 5727 or Union Carbide 10854: m-isopropylphenyl methylcarbamate
IV. Bayer 37344: 4-(methylthio)-3,5-xylyl methylcarbamate
V. Bayer 44646: 4-dimethylamino-m-tolyl methylcarbamate
VI. Zectran: 4-dimethylamino-3,5-xylyl methylcarbamate
VII. Dimetilan or GS-13332: 2-dimethylcarbamyl-3-methylpyrazolyl-(5)-dimethylcarbamate
VIII. Isolan or G 23611: 1-isopropyl-3-methylpyrazolyl-(5)-dimethyl-carbamate

less polar metabolite in the excreta. A resistant (R) house-fly strain was also investigated with and without synergist. Although the R strain was 25 times more resistant to Sevin than the S strain, the strain differences in susceptibility and the effect of the sesamex could not be accounted for on the basis of changes in absorption. However, a quantitative difference in rates of metabolism favoured survival of the R strain, since more of the polar metabolite was excreted and less Sevin remained in the body. Sesamex decreased the excretion of metabolites and increased the retention of unchanged Sevin in the bodies of the R flies in exactly the same manner as with the S flies. Sevin metabolism yielded at least three metabolites in the house-fly, only a very polar metabolite in the milkweed bug (Oncopeltus fasciatus Dall.), and six metabolites in the German cockroach (Blattella germanica L.). Sevin and 1-naphthol formed the same series of more polar products in these insects. The authors concluded that the metabolic pattern differed greatly in the three insects, that the rate of metabolism was related to the toxicity to a particular insect species and to the action of the synergist, and that the rate of formation of one key product probably controlled the overall metabolism. They proposed that the rate of hydrolysis of the ester bond by a carbamate esterase enzyme was the first and rate-limiting step in the metabolism of Sevin.

A recent investigation [23] has established that the metabolism of Sevin is not as simple as a carbamate esterase mediating hydrolysis and other systems then degrading the hydrolysed fragments. It appears instead that oxidative attack by enzymes in liver microsomes, and possibly also insect microsomes, in the presence of the co-factor, reduced nicotinamide-adenine dinucleotide phosphate ($NADPH_2$), may be the initial and critical detoxification mechanism and that hydrolysis of the carbamate ester may or may not follow oxidation.

Sevin was metabolized by rat-liver microsomes to yield at least five different carbamate metabolites with the C-O-C(O)-N-C skeleton intact, and two further metabolites that lacked the carbonyl and methyl group [23]. These conclusions resulted from studies where three samples of Sevin labelled with carbon-14 in the methyl, carbonyl and 1-naphthyl positions were used in separate but identical experiments. Metabolites were separated with column chromatography on Florisil using mixtures of hexane and ether, and on thin-layer chromatographic plates with silica gel, using hexane-ether mixtures. The carbonyl-C^{14}-labelled derivatives were utilized to ascertain suitable conditions for hydrolysis of the carbamate metabolites. Possible modification of the N-methyl group was then studied with metabolites from the methyl-C^{14}-labelled sample by hydrolysis and determination of formaldehyde-C^{14} as 3,3-methylene-C^{14}-bis (4-hydroxycoumarin) after reaction with 4 hydroxycoumarin [24], and of methyl-C^{14}-amine as methyl-C^{14}-phenylthiourea after reaction with phenylisothiocyanate. The structure of these labelled derivatives was verified by both isotopic dilution analysis and co-chromatography on silica-gel thin-layer chromatograms. Ring-labelled metabolites were subjected to hydrolysis and separated on thin-layer chromatograms as the phenolic derivatives or as the methylcarbamates after reaction of the hydrolysis products with methyl isocyanate. These techniques revealed that modifications of both the ring and methyl group

were involved within this series of metabolites. Characterization studies on these Sevin metabolites are in progress.

House-flies and American cockroaches (Periplaneta americana L.) yielded the same series of metabolites as those formed by the rat-liver microsomes, based on their chromatographic characteristics and experiments with the different sites of radiolabelling. Thus insects also formed at least five carbamate metabolites from Sevin [23]. The use of multiple-labelling of the insecticide allowed a more complete evaluation of the metabolism leading to a different interpretation of the metabolic pathway in insects as compared with a study [18] where only Sevin-naphthyl-C^{14} was investigated.

When Sevin has been fed to or sprayed on lactating animals, no significant residues of Sevin or metabolites responding to the p-nitrobenzenediazonium fluoborate chromogenic reagent after hydrolysis have been detected in the milk [25-28]. The complexity of the metabolism of Sevin by liver microsomes prompted the treatment of a lactating goat with carbonyl-C^{14}-Sevin, using a single oral dose of 1.3 mg/kg [23]. A method for the analysis of milk for Sevin and metabolites was developed by fortification of goat milk with the radiolabelled metabolites formed by rat-liver microsomes. The method involved extraction with acetonitrile and chloroform and chromatography of the organosolubles on a Florisil column. Sevin reached a peak level of 0.004 ppm in the milk after 2 h; a metabolite which appears to be a methylcarbamate with the ring modified, probably by hydroxylation, reached a peak level of 0.075 ppm at 2 h; and a metabolite not formed by rat-liver microsomes reached a peak level of 0.027 ppm at 16 h. The level of total Sevin-C^{14}-equivalents appearing as metabolites in milk reached a peak of 0.93 ppm at 8 h and diminished progressively thereafter to below 0.003 ppm by 60 h. The majority of these radiolabelled metabolites were not extractable into organic solvents and may represent conjugates of certain hydroxylation products of Sevin. An analysis of the urine of this goat, which contained about half of the administered radioactivity, revealed the presence of the same metabolites. These results indicate that analysis for free and conjugated 1-naphthol, as for detection of possible occupational or other exposure by urine analysis [28-30] or of possible milk contamination following ingestion of feed containing Sevin residues or direct application of Sevin to animals, may detect only a portion of the metabolites eliminated by these routes.

Sevin does not yield persistent tissue residues in animals exposed to this insecticide, according to analyses of tissue extracts using p-nitrobenzenediazonium fluoborate before and after hydrolysis [31-33]. In order to examine the mechanism of this rapid dissipation of Sevin residues further, the following six compounds (specific activity 1 mc/mM) were administered intraperitoneally to male rats at 7.5 μM/kg: Sevin labelled with carbon-14 in the carbonyl, methyl and 1-naphthyl positions; 1-naphthol-1-C^{14}; methyl-C^{14}-amine hydrochloride; and sodium carbonate-C^{14} [22]. Sodium carbonate yielded rapid (89% within one hour) and almost complete elimination of the radioactivity in the expired air, whereas with Sevin-carbonyl-C^{14} only about one-quarter of the radioactivity was eliminated by this route and the time for half-elimination of that which was expired was about 4 h. With methyl-C^{14}-amine, about half the radioactivity was eliminated as expired carbon-14

dioxide, whereas with Sevin-methyl-C^{14} this value was only about one-eighth of the administered radioactivity, the times for half-elimination of that which was expired being about 1.5 h for methyl-C^{14}-amine and 8 h for Sevin-methyl-C^{14}. No radioactivity appeared as carbon-14 dioxide with Sevin-naphthyl-C^{14} or with 1-naphthol-1-C^{14}. With all six labelled compounds the elimination of radioactivity in the faeces accounted for less than 1% of the administered dose. The radioactivity from Sevin-naphthyl-C^{14} and 1-naphthol-C^{14} was largely eliminated in the urine, leaving very little residual carbon-14 in the tissues after 48 h. The radioactivity in the tissues at 48 h, by which time the rate of elimination of carbon-14 from the rats with all six compounds was negligible, was higher with Sevin-carbonyl-C^{14} than with sodium carbonate-C^{14}, but was lower with Sevin-methyl-C^{14} than with methyl-C^{14}-amine. The total Sevin-C^{14}-equivalents for ten rat tissues ranged from about 0.1 to 0.5 μM/kg 48 h after administration of Sevin-carbonyl-C^{14}, but ranged to 1.7 μM/kg after administration of Sevin-methyl-C^{14}. The radioactivity was most persistent with both carbonyl- and methyl-labelled Sevin in the corpuscles, brain, heart, lung, and particularly the liver and kidney. The localization of the carbon-14 and specific activity of the tissues were quite different with labelled sodium carbonate and methylamine compared to carbonyl- and methyl-labelled Sevin. Since the radioactivity persisting in the tissues was very low for that from the Sevin-naphthyl-C^{14} compared with the other labels, the residual radioactivity was largely due to metabolites lacking the bicyclic ring. Tissue residue differences between administration of carbonyl- and methyl-labelled Sevin and their hydrolysis products may have resulted from variation in distribution of the fat-soluble Sevin compared with its water-soluble hydrolysis products and subsequent localization of metabolites at different sites. Much of the radioactivity (about 60%) appeared in the urine as carbamate metabolites of Sevin, but the identity of these has not been established.

The fate of Sevin after application to plants is incompletely understood. Many studies have been reported on the persistence of this insecticide on plants, based on colorimetric analysis for 1-naphthol after hydrolysis of the insecticide during the analytical procedure (see, e.g. [34-38]). Bean and cotton plants injected through the stem with Sevin-C^{14} slowly converted it to metabolites which could not be extracted into organic solvents from aqueous solution [23]. Five of the carbamate metabolites as detected with animals were not evident in the plants. It is not known whether the major water-soluble metabolite from the plants is the same as that from animals, although this seems unlikely. Little loss of carbon-14 occurred from the plants, even with the carbonyl-C^{14} material and after 28 days, when over 90% of the carbon-14 from Sevin was present as metabolites.

Experiments similar to those conducted with Sevin were also made with o-isopropoxyphenyl methylcarbamate (compound II of Fig. 1) C^{14}-labelled in the carbonyl position [23]. The microsomes of rat liver and the cockroaches yielded two organosoluble metabolites and one or more metabolites which were not readily extracted from water into organic solvents. The principal organosoluble metabolite appeared to be o-isopropoxyphenyl N-methylolcarbamate based on isolation, degradation and synthesis. The behaviour of o-isopropoxyphenyl methylcarbamate in plants was similar to that of Sevin.

Only limited information is available on the biological activity of these metabolites of the methylcarbamates. The organosoluble metabolites from the liver microsomes of 1-naphthyl and o-isopropoxyphenyl methylcarbamates were less than one-eight as potent as their methylcarbamate precursors when assayed in vitro for anticholinesterase activity with fly-head homogenates [23].

3. METABOLISM OF N,N-DIMETHYLCARBAMATES

The metabolism of Dimetilan-5-carbonyl-C^{14} (VII) was investigated in adult American and German cockroaches [39]. The half-life of the injected compound in the German cockroach was less than one hour, with the loss occurring from elimination as carbon-14 dioxide and formation of at least three organosoluble C^{14}-metabolites, none of which was 3-methylpyrazolyl-(5)-dimethylcarbamate. The metabolites in the American cockroach were examined in more detail using both thin-layer chromatography on silica gel with an ethyl acetate-ethanol (98:2) developing solvent, and column partition chromatography with silica gel developed with hexane-methylene chloride mixtures. Chromatography on the silica-gel column of an extract prepared from roaches 4 h after injection of 1,0 mg Dimetilan-C^{14} per roach resulted in recovery of about half of the original carbon-14 injected. About one-fourth of the injected dose was recovered as Dimetilan, and one-fourth as four metabolites which were partially resolved on the column and more completely so on the thin-layer chromatograms. Two of these metabolites yielded formaldehyde on acid degradation. The biological activity of these metabolites was less than that of the Dimetilan precursor on the basis both of toxicity to house-flies and of anticholinesterase activity as assayed in vitro with house-fly head acetylcholinesterase and human plasma butyrylcholinesterase. Infra-red spectra on the two major metabolites, those which yielded formaldehyde on acid degradation, were consistent with the presence of both carbamyl groups on the molecule, but with an alcoholic function which might result from the oxidation of a methyl to a methylol group. On the analogy of the metabolism of similar non-labelled compounds by rat-liver microsomes and insects [40, 41] it would appear likely that the formaldehyde-yielding metabolites from Dimetilan were N-methylol derivatives.

Mammals treated with Pyramat [2-propyl-4-methylpyrimidinyl-(6)-dimethylcarbamate] excreted three metabolites in the urine. One was identified as 2-propyl-4-methyl-6-hydroxypyrimidine and the others were presumed from limited evidence to be 2-propyl-4-methyl-5,6-dihydroxypyrimidine and a carbamate intermediate which might decompose to yield this latter enol [42]. Metabolism of this dimethylcarbamate insecticide thus appeared to involve ring hydroxylation, but methyl hydroxylation and some conjugation may also have occurred as acid hydrolysis increased the recovery of certain of the enolic products.

4. OTHER RADIOTRACER APPROACHES

The availability of methods of preparing C^{14}-labelled carbamates with specific activities of 1 to 5 mc/mM should facilitate, and perhaps even

stimulate, further critical investigations on the mode of action of this relatively new group of insecticides.

The insecticidal activity of the methylcarbamates is known to be related at least in part to their rate of metabolism by insects based on many types of experimental evidence, including a single study with radiolabelled compounds [18]. Species specificity in insecticidal activity may be a function of differences in rate of metabolism of the insecticides. Resistance acquired to the carbamates in strains pressured with agents of this type, or as a side-resistance where the insects were selected with non-carbamate compounds, appears to be related to the greater ability of the resistant strain to detoxify the carbamate. The action of methylenedioxyphenyl compounds and others that serve to synergize the insecticidal activity of the carbamates is probably associated with decreasing the efficiency of detoxification of the toxicants. Certain of these synergists may act by inhibiting the microsome-$NADPH_2$ system involved in hydroxylation of the N-methyl groups of the enolic portion of the molecule. The experimental evidence supporting these relationships has been recently reviewed [1, 2, 5, 18, 43, 44]. The ultimate testing of these hypotheses requires detailed knowledge of the rates of the various detoxification reactions in the appropriate insects. Radiotracer and chromatographic techniques adequate for such studies are now available.

Detoxification rates may also be related to certain aspects of the toxicity to mammals of the methylcarbamates, although adequate experimental evidence is not yet available on this points. Insecticidal carbamates vary in rat oral LD50 from less than 1 to more than 1000 mg/kg, although most of the compounds being considered for development as commercial insecticides fall in the range 10–500 mg/kg. The route of administration may have a large and differential effect on the toxicity, as illustrated by Isolan (VIII), which is more toxic to mammals following dermal than oral administration, and Dimetilan (VII), where the dermal is much less than the oral toxicity. Toxicity changes with different substituents on the molecule and with different routes of administration may be related in part to the stability of the compound in the body. With sufficient information on the relationship of structure to rates of metabolism of the carbamates in different organisms, it may be possible to design a molecule that would be readily detoxified by mammals and less readily decomposed by insects. This appears to be the case with Sevin, which has a considerable level of selectivity in its toxic action. This selectivity might be further increased by the use of suitable adjuvants, such as the methylenedioxyphenyl and other synergists, to reduce further the detoxification rate by insects without affecting the rate by mammals. The fundamental information leading to such approaches to selective toxicity will probably come in large part from radiotracer studies.

Radiotagged compounds have been extremely valuable in determining the distribution of pesticides among tissue and organ systems in plants and animals. Considerable fractionation will be necessary to define the localization of the original radiolabelled carbamate in insects and mammals, since metabolites of somewhat similar solubility properties are rapidly formed. The carbamates which have been investigated, by either radiotracer or colorimetric techniques, are not stored for prolonged periods

in any particular depots. Many ionized carbamates are almost inactive as insecticides, despite very high anticholinesterase activity, presumably because of their inability to penetrate into the insect nervous system at a sufficient rate to achieve the critical levels necessary for blocking impulse conduction. Data on the level of intact carbamate or its anticholinesterase metabolites in the insect nervous system might be advantageously compared with the degree of cholinesterase inhibition occurring within a portion of the nervous system, such as the thoracic ganglia or entire nerve cord. The correlation of the actual level of carbamate in the nervous system with the symptoms and degree of inhibition of acetylcholinesterase and other carbamate-sensitive esterases, such as aliesterases, would contribute to an evaluation of the mode of insecticidal action of these materials. Further studies are needed on the penetration rate into insects as affected by formulation and other adjuvants, and the rate of absorption and distribution following ingestion, particularly since considerable variation occurs in insecticidal activity depending on whether the compounds are applied topically, injected or ingested. Penetration and distribution studies in plants utilizing methylcarbamates-C^{14} might be more readily approached than similar studies in animals, since the compounds studied so far are more stable in plants than animals and the plant metabolites vary greatly from the original compound in solubility characteristics.

Studies on the residual persistence of the carbamate insecticides have been made primarily by determination of the enolic or amine portion of the molecule as liberated by hydrolysis during analysis. The validity of any such method depends on the specificity in accounting for all toxic agents, with minimum interference from non-toxic metabolites. It is known that metabolism may modify both the amine and enolic portions of the molecule, and that certain of the metabolites are biologically active. Metabolism involves both oxidation and hydrolysis, with or without conjugation of certain of the hydroxylated derivatives. This same situation also occurs with other pharmacologically active but non-insecticidal carbamates [45]. Ethyl carbamate (urethane) as studied with a carbonyl-C^{14}-label and probably also methyl carbamate are rapidly hydrolysed in mammals [46-48]. Ethyl chloramate [ethyl 2,2,2-trichloro-1-hydroxyethylcarbamate] is partially hydrolysed and partially conjugated [49]. Mephenesin carbamate (3-o-tolyloxy-1,2-propanediol 1-carbamate) is quite stable in mammals, and its metabolism includes conjugation of a portion of the dose without hydrolysis of the carbamate ester group [50, 51]. In vivo hydrolysis of methocarbamol [3-(o-methoxyphenoxy)-1, 2-propanediol 1-carbamate] is negligible, according to a carbonyl-C^{14} study, and the excreted metabolites include the original compound and its glucuronide and four further metabolites, two of which are glucuronides [52]. Meprobamate (2-methyl-2-n-propyl-1, 3-propanediol dicarbamate) labelled with both tritium (Wilzbach technique) and carbon-14 has been investigated and its metabolism found to involve almost no hydrolysis but rather predominantly hydroxylation of the 2-methyl position with or without glucuronide formation before excretion [53, 54]. Metabolism of both 1-ethynylcyclohexyl carbamate-carbonyl-C^{14} and 1-ethylcyclohexyl carbamate-carbonyl-C^{14} involves ring hydroxylation. Hydrolytic cleavage was a minor catabolic route and excretion occurred as both ring-hydroxylated 1-ethynylcyclohexyl carbamate and its glucuronide [16]. The nature of the changes

in the enolic portion of the methylcarbamate insecticides during metabolism will determine the choice of the phenolic chromogenic reagent to differentiate only the toxic products. Radiotagged metabolites can be separated and the specificity of the cromogenic reagents can be used to assist in elucidating their structure and in selecting an appropriate method for residue analysis. The biological activity of the metabolites can be determined readily with labelled materials, as they need not be completely pure since their amount can be precisely determined on the basis of their initial specific activity.

Toxic manifestations with the carbamate insecticides, as with the organophosphate insecticides, presumably result from inhibition of nerve acetylcholinesterase activity. They differ, however, in the nature of the reaction with the cholinesterase, since the organophosphate inhibition by phosphorylation is only very slowly reversible, whereas the more ready reversibility of the carbamate inhibitors may lead to enzyme-inhibitor dissociation during tissue preparation and assay. The reaction of the carbamate with the esterase is complex, the rates of formation of the enzyme-inhibitor complex, carbamylation of the enzyme and hydrolysis of the carbamylated enzyme being affected by concentration of inhibitor, enzyme and substrate. The sequence of reactions at the esteratic site on the enzyme surface appears to be the same for the substrate and the carbamate and phosphate inhibitors, with the marked differences with these compounds resulting from variations in rate of different components of the reaction. Radiolabelled carbamates are now available with adequately high specific activity for use in studies on the nature of the reaction of the enzyme and inhibitor, an approach allowing possible verification and extension of the kinetic studies which have led to the information now available. Highly purified cholinesterase, from mammalian or insect sources, might be reacted with the radiolabelled carbamates to ascertain whether, for example, a radiolabel on the enolic moiety would be released and the radiolabelled methyl and carbonyl groups would be bound to the protein. The stability of the C^{14}-methylcarbamyl group at the esteratic site of the esterase and the nature of the peptide chain involved at the site of carbamylation might be investigated on similar lines to those adopted for P^{32}-phosphorylated esterases, using P^{32}-organophosphate inhibitors, with the exception that suitable conditions would have to be utilized to prevent spontaneous hydrolysis of the carbamyl group from the esterase or peptide during the studies (for reviews and recent articles relating to this problem see [1, 55-66]). Certain esterases other than cholinesterase have also been observed to hydrolyse some insecticidal carbamates, such as esterases of rabbit plasma, liver and kidney, which catalyse the hydrolysis of dimethylcarbamyl fluoride, and the albumin fraction of mammalian plasmata which increases the hydrolysis rate of certain methylcarbamates, including Sevin [67-69]. These esterases presumably differ from those that are more sensitive to inhibition by carbamates in that the carbamyl esterases are more rapidly hydrolysed, an assumption susceptible to direct test with radiolabelled carbamate substrates. A recent proposal [10] to use acetyl-C^{14}-choline as the substrate in studying the cholinesterase inhibition from carbamates is particularly attractive as a means of minimizing enzyme and inhibitor dilution during assay and utilizing much lower substrate concentrations during analysis than has been possible with other procedures. This should result in

higher observed levels of inhibition. The degree of inhibition for tissue from carbamate-poisoned animals might then more closely approximate, although certainly not reproduce, the in vivo situation (for considerations related to this point see [1, 71-73]).

The labelled-pool technique, using phosphorus-32 and carbon-14 biochemicals, has proved very valuable in considering the mode of action of such insecticides as DDT, dieldrin and others (for review see [74]). No such studies have been reported as yet with animals poisoned with carbamate insecticides. The procedure for examining the rate of acetate-C^{14} incorporation into C^{14}-acetylcholine would be of particular interest, especially if applied to the insect nervous system.

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DISCUSSION

M. HASCOËT: What solvent system did you use for thin-layer chromatography?

J.E. CASIDA: We have a mixture of about half-a-dozen different solvent materials which works very well. Hexane-ether mixtures of varying proportion give good results, the proportion depending on the type of metabolites being used. We usually run these as two-dimensional thin-layer chromatograms in order to determine the stability of the metabolites on the thin layer and we run them with each radio-label.

M. HASCOËT: What is the capacity of the Florisil column that you use? How many milligrams per gram do you need to obtain a good resolution?

J.E. CASIDA: The capacity is about 50-150 mg. For further information I would refer you to my reference [19]. Actually you cannot obtain as good a resolution by this means as with thin-layer chromatography – this is the experience of many people who have tried both. You can probably run at about 5 mg/g but this is a top level. Optimum resolution would be obtained with about 1 mg/g.

M. HASCOËT: Is there no degradation of Sevin on the column?

J.E. CASIDA: This has been very carefully checked in a variety of experiments. There is no degradation on the column, but there is degradation for some of the metabolites if left for prolonged periods on the silica-gel thin-layer chromatogram.

M. HASCOËT: At what temperature is the silica gel activated?

J.E. CASIDA: Our silica gel is obtained in a large drum and we take it from the drum and add water. We do not activate it. With each new batch of silica gel we re-ascertain the optimal conditions for separating the material. These conditions, like the purity of the solvent, appear to play a large part in determining the quality of resolution obtained.

C.E. SEKERIS: With thin-layer chromatography I think you are able to see how many hydroxyl groups you have on your ring, if you choose the right elution system.

J.E. CASIDA: Yes. We have been using it in this manner to clarify the modifications occurring on the naphthol ring.

H. HUQUE: Insects are said to develop resistance very quickly in the case of Sevin. What are the factors responsible for this?

J.E. CASIDA: My references [1], [4] and [5] provide information on this point. The rate of development of resistance depends on the slope of the dosage-mortality curve, the type and level of field application made and the previous history of exposure of the insects in question to other insecticides. House-flies quickly develop resistance to Sevin, but the Mexican bean beetle does not. Synergists may reduce the rate of developing re-

sistance. The rate of metabolism of Sevin appears to be at least partially correlated with resistance in selected strains and this presumably results primarily from in vivo hydroxylation, as discussed. The techniques are now available for the critical studies on resistance which are needed.

H. I. PETERSEN: A problem of very great interest in this connection is the toxicity of the metabolites. Can you say anything about this?

J. E. CASIDA: The experiments that we ran were limited because we had a limited supply of metabolites. However, with Dimetilan it is very easy because we can put 1 mg into each cockroach and recover about 250 μg as pure metabolite by passing through one column – it will then crystallize so that we can get hundreds of milligrams of these metabolites. None of them are as potent as Sevin in inhibiting cholinesterase from its source in the house-fly brain. The anti-cholinesterase activity of the materials is, of course, no indication of their toxicity, and until we can make enough of these materials to study their toxicology independently the question remains unresolved. I think it is an important question because they appear in quite a number of foodstuffs produced by currently recommended procedures.

C. C. HASSETT (Chairman): Dr. Casida, would you care to comment on problems of cross-resistance with the carbamate insecticides?

J. E. CASIDA: I believe Dr. Meltzer investigated this rather closely. I think it would be more appropriate for him to answer.

J. MELTZER: We have had a lot of experience in the selection of strains of house-flies using phenyl-dimethyl-carbamate. Within a certain strain of house-flies, this compound induced a remarkable cross-resistance, in particular to chlorinated hydrocarbon insecticides, whereas the resistance to the carbamate was only slight. Work with other strains of house-flies showed that it was possible to obtain resistance to the phenyl-dimethyl-carbamate, but the maximum resistance did not exceed a factor of 10.

I should like to add, however, that our work with selections of resistant strains showed that we should be very careful in generalizing results. If we obtain a certain result by our selections, we can only say that the results apply to the particular strain used, and under the particular circumstances. Under other conditions, we might obtain contradictory results. I would, therefore, like to warn against generalizing the results obtained in this selection work.

J. E. CASIDA: I would like to make one final comment on this question of resistance, drawing attention in this connection to references [1], [4] and [5] to my paper. From the studies that have been made so far it appears that metabolism in insects is not by hydrolysis but by oxidation. There is considerable evidence in the literature that resistance is related to the rate of metabolism, so that one could deduce that the resistance is due to an increased ability to carry out this oxidation. There is no proof of this, however.

PROBLEMS OF APPLICATION AND ACTION OF THIODAN STUDIED WITH S^{35}-LABELLED INSECTICIDE

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Abstract — Résumé — Аннотация — Resumen

PROBLEMS OF APPLICATION AND ACTION OF THIODAN STUDIED WITH S^{35}- LABELLED INSECTICIDE. Thiodan (6,7,8,10,10-hexachloro - 1,5,5a,6,9,9a - hexahydro-6-9-methano -2,4,3 - benzodioxathiepin - 3 - oxide) is an insecticide developed by Farbwerke Hoechst AG. The technical product consists of two isomers with different melting points and different velocities of insecticidal effect. Using highly purified X- and S-isomers together with technical product, all labelled with S^{35} of the same specific activity, the authors studied problems of the application and mode of action of the isomers. Since the insecticide can be experimentally applied in the vapour phase, we tested the effect of different temperatures and air humidities on sublimation on the end penetration through the cuticula. As intoxication began, a reactive increase in respiration, followed by a remarkable elevation of the insects' body temperature, was found. It was shown with the labelled insecticides that this raising of body temperature after some time causes surface removal of the sublimated insecticidal substance by way of resublimation into the air. This mechanism influences the complex mechanism of penetration, intoxication and detoxication in connection with temperature and relative humidity of the air.

Further experiments are concerned with the penetration and distribution of the labelled insecticide in the insect organism.

MODES D'APPLICATION ET D'ACTION DU THIODAN ÉTUDIÉS PAR MARQUAGE DE L'INSECTICIDE PAR ^{35}S. Le Thiodan (6,7,8,10,10-hexachloro - 1,5,5a,6,9,9a - hexahydro-6-9-méthano -2,4,3 - benzodioxathiépine - 3 - oxyde) est un insecticide mis au point par la Farbwerke Hoechst Ag. Le produit fabriqué comprend deux isomères ayant des points de fusion différents et dont l'effet insecticide ne se manifeste pas avec la même rapidité. En utilisant simultanément les isomères α et β à l'état très pur et le produit fini, tous trois marqués par ^{35}S de même activité spécifique, on a étudié les problèmes relatifs au mode d'application du produit et au mode d'action des isomères. Comme l'insecticide peut être appliqué expérimentalement en phase vapeur, on a vérifié l'effet de diverses températures et de divers degrés d'humidité de l'air sur la sublimation à la surface de la cuticule et sur la pénétration à travers celle-ci. On a constaté que le début d'intoxication provoquait une accélération du rythme de la respiration, suivie d'une élévation sensible de la température du corps des insectes. Comme on a pu le constater en utilisant des insecticides marqués, cette augmentation de la température du corps provoque, après un certain délai, la disparition de l'insecticide sublimé à la surface, qui s'évapore de nouveau dans l'air. Ce phénomène influe sur le mécanisme complexe de la pénétration, de l'intoxication et de la désintoxication en fonction de la température et de l'humidité relative de l'air.

D'autres expériences ont porté sur la pénétration et la distribution de l'insecticide marqué dans l'organisme de l'insecte.

ПРОБЛЕМЫ ПРИМЕНЕНИЯ И ИЗУЧЕНИЕ СПОСОБА ДЕЙСТВИЯ ТИОДАНА ПРИ ПОМОЩИ МЕЧЕННЫХ S^{35} ИНСЕКТИЦИДОВ. Тиодан (6, 7, 8, 10, 10-гексахлоро-1, 5, 5а, 6, 9, 9а-гексагидро-6-9-метано-2, 4, 3-бензодиоксатиепин-3-оксид) - инсектицид, выработанный фирмой "Фарбверке Хехст АГ". Этот технический продукт состоит из двух изомеров с различными температурами плавления и с различной скоростью инсектицидного эффекта. Используя высокоочищенные альфа- и бета-изомеры и технический продукт, меченный S^{35} одинаковой удельной активности, удалось изучить проблемы применения и способы действия этих изомеров. Так как этот инсектицид может быть экспериментально применен в газообразном состоянии, авторы проследили за влиянием изменений температуры и влажности воздуха на сублимацию и проникновение инсектицида через кутикулу. Одновременно с начинающимся отравлением авторы обнаружили реакцию, выражающуюся в учащении дыхания, вслед за чем наблюдалось значитель-

ное повышение температуры тела насекомых. Как установлено при помощи меченых инсектицидов, это повышение температуры тела вызывает через некоторое время исчезновение сублимированного инсектицидного вещества с поверхности путем обратной сублимации в воздух. Этот процесс влияет на сложный механизм проникновения, отравления и устранения токсичности в зависимости от температуры и относительной влажности воздуха.

В дальнейших экспериментах рассматривается проникновение и распределение меченого инсектицида в организм насекомого.

ESTUDIO DE LA APLICACION Y DEL MODO DE ACTUAR DEL THIODAN MARCADO CON ^{35}S. El Thiodan (6,7,8,10,10-hexacloro - 1,5,5a,6,9,9a - hexahidro-6-9-metano -2,4,3 - benzodioxatiepina-3-óxido) es un insecticida preparado por la Farbwerke Hoechst AG. El producto comercial consiste en dos isómeros de diferente punto de fusión, que actúan con velocidades distintas. El autor de la memoria ha estudiado los problemas que plantean la aplicación y el modo de actuar de los isómeros empleando los isómeros α y β de elevado grado de pureza junto con el producto comercial, todos ellos marcados con ^{35}S de la misma actividad específica. Como el insecticida puede aplicarse experimentalmente en la fase vapor, se comprobó el efecto de la temperatura y de la humedad del aire sobre la sublimación y la penetración a través de la cutícula. Al comenzar la intoxicación se observó un aumento del ritmo respiratorio seguido de una notable elevación de la temperatura del cuerpo de los insectos. Los insecticidas marcados permitieron demostrar que este incremento de la temperatura se produce algo después de que el insecticida condensado en la cutícula ha podido comenzar a evaporarse. Este proceso ejerce influencia sobre el complejo mecanismo de la penetración, intoxicación y desintoxicación, y depende a su vez de la temperatura y de la humedad relativa del aire.

Se efectuaron nuevos experimentos para estudiar la penetración y la distribución del insecticida marcado en el organismo del insecto.

INTRODUCTION

Thiodan® is an insecticide developed by Farbwerke Hoechst AG, Federal Republic of Germany [1-2]. The commercial product (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2,4,3-benzodioxathiepin-3-oxide) consists of two isomers with distinctly different melting points and different rates of insecticidal action [3]. The configuration of the stereoisomers was studied by RIEMSCHNEIDER et al. [5]; in this paper they are designated as the α-isomer (F = 108-110°C) and the β-isomer (F = 208-210°C). As shown in Fig. 1, the Thiodan molecule was labelled with radiosulphur (S^{35}); the

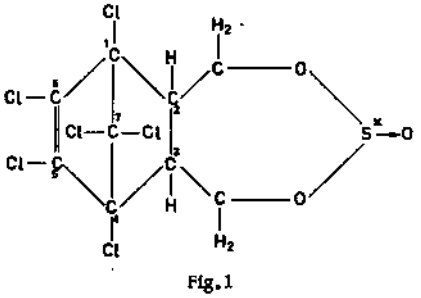

Fig. 1

Molecular structure of Thiodan-S^{35}

highly purified α- and β-isomers and the commercial product (F = 103°C) had the same specific activity (20.6 mc/g). It is generally postulated that a modern synthetic insecticide must possess two essential properties: first, it must be toxic to the insect and secondly it must be able to gain access to

its specific site of action either by penetration through the cuticle or by other paths. In this case, tracer methods are an excellent way of studying the problems of pick-up and penetration. This paper gives a brief account of a large field of experiments using tracer techniques as a part of complex investigations into the mode of action of Thiodan [3, 4, 6].

PICK-UP OF THIODAN BY THE SURFACE OF THE INTEGUMENT AND THE INFLUENCE OF RELATIVE AIR HUMIDITY

The first part of the work to be described attempts to answer the question: Do the varying relative humidities of the air influence the pick-up of Thiodan and its two isomers? Among the different application methods for testing insecticides, the use of the vapour phase is advantageous in our case, because the test insects are exposed to the pure insecticide in molecular form. The main experiments have been done with the granary weevil Calandra granaria L. on account of its relatively large body surface and small amount of body fat. The insects were kept in closed hygrostatic dishes with an inset made of copper gauze on which they were able to run about (Fig. 2). The bottoms of the dishes were filled with super-saturated inorganic salt

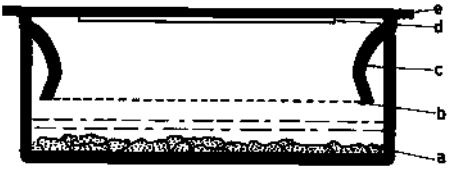

Fig. 2

Special hygrostatic dish for application of radio-Thiodan under controlled air humidity (9 cm diameter)
a = supersaturated salt solution
b = copper gauze
c = inset
d = filter paper impregnated with insecticide
e = glass lid

solutions providing the different relative humidities (K_2SO_4 = 98-100% r. h., Na Cl = 74% r. h., $Ca(NO_3)_2$ = 54% r. h., $Zn Cl_2$ = 17% r. h.).

Filter papers of 7-cm diameter had been previously impregnated with 1 ml of a Thiodan-acetone solution (500μg Thiodan) and were fixed under the glass lid after evaporation of the solvent. This method permitted the molecular insecticide particles to come into contact with the entire surface of the weevils from all directions. The exposure was done in a constant test temperature of 30°C. After 72 h 50 granary weevils in each case were collected from the dishes and washed five times with 2 ml cold methanol by means of a sprayer. By this means all the insecticide picked up was removed from the superficial layers. We preferred methanol to ether and chloroform for removing Thiodan from outside the insect, since, according to BEAMENT [7], it is a poor solvent for the wax of the insect cuticle. The five fractions were collected in small aluminium planchets. After evaporation of the solvent, they were measured for 10 min by means of a Frieseke

and Hoepfner electronic scaler in conjunction with an end-window counting-tube having a window thickness of 1.2 - 1.3 mg/cm².

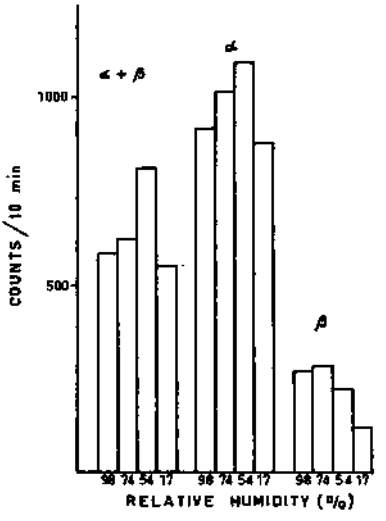

Fig. 3

Amount of Thiodan-S^{35} isomers picked up by 50 granary weevils exposed (72 h, 30°C) to the vapour phase of the insecticide under different air humidities

The first point of interest in Fig. 3 is that different amounts of Thiodan and its α- and β-isomers were picked up by the superficial layers of the integument. This had been anticipated, because the difference in the quantity picked up depends on physical properties, i.e. the different melting points of the α- and β-isomer, as well as on the composition of the commercial product ($\alpha:\beta = 3:1$) [8].

Further, it is apparent – and this is the main point of interest – that the pick-up of Thiodan on the epicuticle is considerably influenced by the relative humidity. These effects are likewise due to physico-chemical properties of the superficial layers. As the wax layers of the insect epicuticle consist of mixtures of paraffins, alcohols and fatty acids, i.e. a mixture of lipoids of varying reactivity [9], it has a certain solubility for Thiodan, which is a lipophilic insecticide. If a Thiodan molecule comes into contact with the epicuticle the surface and the particle will be subjected to attractive forces varying in intensity from primary valence bonds through hydrogen bonds to long-range intermolecular or van der Waals forces. The highest rate of solubility is shown in the middle range of relative humidities, at which the wax layer is not covered with a thin film of water and the transpiration rate is relatively low. In this case, we assume that the dissolving forces of the epicuticle are fully effective. At high humidities, a thin film of water covers the epicuticle, which reduces the solubility for the insecticide, while at very low humidities the rate of transpiration and loss of water through the cuticle act against the deposition and pick-up of the Thiodan molecules [6].

THE PICK-UP AND PENETRATION OF THIODAN AND ITS ISOMERS

In the second series of experiments we tried to clarify the problem of the relationship between pick-up and penetration through the integument and the symptomatology of the insecticidal action.

These studies were done with the same experimental arrangement as described above, but now the granary weevils (50 specimens) were exposed directly to deposits of the pure isomers or commercial product on filter paper impregnated with insecticide to 0.63μg/cm^2 for varying periods at 25°C and 54% r.h. After different periods the symptoms induced by the insecticide were evaluated. For the purposes of the following presentation the symptoms will be designated by numerals: 1 = latency phase; 2 = incipient excitation; 3 = strong excitation; 4 = convulsions; 5 = paralysis and death.

The weevils were collected and first of all washed five times with 2 ml cold methanol. This removed the Thiodan collected on the surface for determination. This fraction is termed the "outside" fraction. After this, the test insects were homogenized in a Potter-Evelhjem homogenizer with 5 times 2 ml acetone. This fraction is termed the "inside" fraction. Since the Thiodan was labelled with S^{35}, combustion and ash measurement was not a convenient procedure; it was therefore necessary to establish a standard curve for the absorption of β-radiation by the body substances which are also extracted. The amount of fat extracted from 50 granary weevils absorbs at least 31% of the β-radiation.

As Fig. 4 shows, different amounts of Thiodan and its isomers had been picked up by the insects from deposits of equal density during equal periods. If the pick-up had been no more than superficial contamination, it might

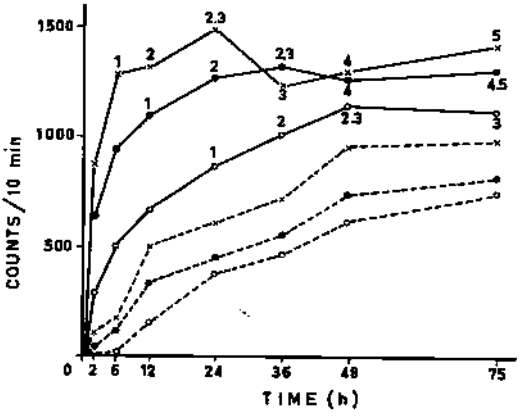

Fig. 4

The pick-up and penetration of Thiodan-S^{35} and its isomers in relation to the increasing insecticidal effect (designated by numerals 1–5 as described in the text)

Outside	Inside
X —— X α	X - - - X α
O —— O β	O - - - O β
● —— ● α + β	● - - - ● α + β

have been expected that the amounts of the three substances recovered in the "outside" fraction would have been equal. Since this is clearly not the case, however, it demonstrates that there must be differential solubility for the three substances in the epicuticle, depending on their different physico-chemical properties, in particular their stereoconfiguration [6]. These facts are confirmed if one also compares the times at which the visible symptoms evoked by the poison appear: For α-Thiodan, the stage of irreversible injury (symptoms 2 and 3) is reached after 24 h and for commercial Thiodan after 36 h, whereas for the β-isomer this stage is not reached for 48 h. It must be emphasized that all three substances are absolutely toxic for insects [3, 6]; the better initial toxicity of the α-isomer is merely a function of its physico-chemical properties. By comparing the symptoms induced by Thiodan intoxication and the amount of "outside" pick-up, it may be seen that up to the end of the incipient excitation phase (symptom 2) and beginning of the strong excitation (symptom 3) insecticide is picked up steadily and the curve has a distinct peak. During the strong excitation phase, however, no more insecticide is picked up; in fact the detectable activity of the outside falls. At a later stage (symptom 4) pick-up again rises. ARMSTRONG et al. [10], working with the isomers of benzene hexachloride, obtained curves of nearly the same type, but were unable to explain this effect. By means of an apparatus which enables us to measure the body temperature in the body cavities of insects [3, 6], we found that the temperature rises from incipient excitation until the end of the strong excitation (symptoms 2 and 3). Parallel to the rise in body temperature, the oxygen consumption curve also has its peak at the same intoxication stages [6].

Another experiment to confirm the fall of the outside curves after intoxication stages 2 and 3 was made by subjecting life weevils and freshly killed weevils under identical experimental conditions to radioactive Thiodan vapour. It was found that the pick-up of labelled Thiodan showed a linear increase in the dead weevils, whereas the live weevils demonstrated the typical fall of activity during excitation stage 3 [6]. These experiments represent an absolute proof that the fall of the "outside" curve is due to a physiological reaction on the part of the insects.

If one examines the shape of the "inside" curves it appears that the α-isomer penetrates the insect more rapidly than the commercial product and the β-isomer. After two hours' exposure, the α-isomer and commercial product already appear in substantial amounts "inside" the insects. Thus it is confirmed that the structural difference between the isomers and the quantitative composition of commercial Thiodan have a considerable effect on the ability to penetrate the insect cuticle [6]. Allowing for the different amounts of "outside" pick-up, the "inside" curves show nearly the same shape. There is a visible increase from the latency phase 1 through the incipient excitation stage 2, whereas during the strong excitation stage 3 the rise of the curves is very small. As we have stated before, all three substances possess absolute toxicity; a total amount of 0.4 μg of Thiodan or its isomers must be present "inside" the insects to cause irreversible injury in 50 granary weevils.

It has been shown, by working with the labelled insecticide, that Thiodan possesses the postulated properties of an active insecticide; it has an absolute and sufficient toxicity for the insect (0.008 μg per insect will kill granary weevils in 100% of the cases) and the ability to gain access to its specific site of action.

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DISCUSSION

B. DARIS: Did you count the number of dead weevils in different relative humidities after the period of 72 h?

W. KLOFT: Yes, naturally we did that, but the results will be published elsewhere.

B. DARIS: I am especially interested in the high relative humidity of 98-100%. Did you find live weevils at these high relative humidities?

W. KLOFT: Yes, even at 98-100% r. h. some weevils survive, depending on the insecticide concentration and on the exposure periods. The purpose of the experiments was in fact to find the best concentrations and exposure periods at different relative humidities, in order to establish control methods adapted to particular climatic conditions. We think that so far insufficient attention has been paid to this.

D.A. CROSSLEY: What method do you use to measure respiration for these insects?

W. KLOFT: We measured respiration using Warburg manometric techniques at different temperatures. The insects were put directly into the vessels and were intoxicated – this is important, I feel – within the vessel. First, the rate of oxygen consumption was measured for two hours, and then the respiratory quotient was measured throughout the whole course of intoxication until death.

C.T. LEWIS: I was very interested in the figures for the uptake of insecticide from the vapour phase. I wonder whether you have any information, or any views, on the possibility that sublimated insecticide might have been picked up by the weevils from the gauze on which they were walking. Do you agree that a significant fraction of the uptake might have come from insecticide that had condensed on the gauze surface?

W. KLOFT: Yes, we cannot totally exclude this factor. Only at the beginning of the exposure was the uptake exclusively from the vapour phase, and I am sure that after about two hours we had a supplementary intoxication of the type you mention.

F. T. PHILLIPS: Your results on the pick-up of Thiodan vapour by insect epicuticle under different relative humidities are interesting. Did you use other surfaces for comparison, for example a glass surface (as a standard surface) and a glass surface covered with a layer of insect wax? I realize that it probably would not be possible to obtain the same wax structure on a glass surface as on insect bodies, but this might be overcome by using excised cuticle from the insects.

W. KLOFT: We have not done any experiments exposing glass surfaces under different relative humidities, since we are of the opinion that the differences observed are mainly due to physiological processes, for the reasons explained in greater detail in the paper. In particular, the loss of "outside" activity after a rise in internal body temperature shows that physiological processes are involved, and this was proved by the simultaneous exposure of dead weevils. We were also able to show this resublimation effect using a glass model heated inside by an electric resistance.

E. HORBER: Is there no interaction to be expected between the insecticide vapour and the ions of the solutions in the bottom of the Zwölfer hygrostatic dishes?

W. KLOFT: No, because Thiodan is practically water-insoluble. In addition, the filter with the insecticide is fixed under the lid and the vapour phase comes from above, while the aqueous salt solution is underneath.

ПРИМЕНЕНИЕ РАДИОАКТИВНЫХ ИЗОТОПОВ В ИЗУЧЕНИИ ПРОЦЕССОВ ВСАСЫВАНИЯ, РАСПРЕДЕЛЕНИЯ И ВЫДЕЛЕНИЯ ИЗ ЖИВОТНОГО ОРГАНИЗМА НЕКОТОРЫХ ИНСЕКТИЦИДОВ

Г.В. ФИЛАТОВ, П.А. КАРТАШОВ, М.И. МУТИН, И.А. ЗАКАМЫРДИН, У.Я. УЗАКОВ
ВСЕСОЮЗНЫЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКИЙ ИНСТИТУТ ВЕТЕРИНАРНОЙ САНИТАЦИИ, МОСКВА, СССР

Abstract — Résumé — Аннотация — Resumen

THE USE OF RADIOISOTOPES TO STUDY THE ABSORPTION, DISTRIBUTION AND ELIMINATION OF VARIOUS INSECTICIDES IN ANIMALS. When insecticides are used against farm-animal parasites it is important to ensure that no harm is done to the health of the animal or the consumer. Radioisotopes provide a means of studying the behaviour of labelled insecticides in animal organisms and of obtaining extremely accurate data on residues of insecticides and insecticide decomposition products in meat and milk.

The paper gives details on the rate at which DDT-C^{14}, polychloropinene-Cl^{36} and chlorophos-P^{32} are absorbed through the skin, accumulated in the organs and tissues and eliminated from the organisms of farm and laboratory animals.

EMPLOI DES RADIOISOTOPES POUR L'ÉTUDE DES PROCESSUS D'ABSORPTION, DE DISTRIBUTION ET D'EXCRÉTION DE CERTAINS INSECTICIDES DANS L'ORGANISME VIVANT. L'emploi des insecticides dans la lutte contre les parasites des animaux de ferme ne doit pas compromettre la santé des animaux ni celle des personnes utilisant les produits animaux. Les radioisotopes permettent de suivre le comportement d'un insecticide marqué qui a été introduit dans l'organisme animal, et de déterminer par ailleurs, avec une grande précision, la teneur du lait et de la viande en insecticides et produits de désintégration.

Le mémoire fournit des données sur la vitesse d'absorption à travers l'épiderme, l'accumulation dans les organes et tissus et l'excrétion, par l'organisme d'animaux de laboratoire et d'animaux de ferme, de DDT-^{14}C, de polychlorure de pinène-^{36}C et de chlorure de phosphore-^{32}P.

ПРИМЕНЕНИЕ РАДИОАКТИВНЫХ ИЗОТОПОВ В ИЗУЧЕНИИ ПРОЦЕССОВ ВСАСЫВАНИЯ, РАСПРЕДЕЛЕНИЯ И ВЫДЕЛЕНИЯ ИЗ ЖИВОТНОГО ОРГАНИЗМА НЕКОТОРЫХ ИНСЕКТИЦИДОВ. Применение инсектицидов для борьбы с паразитами сельскохозяйственных животных не должно наносить ущерб здоровью как самих животных, так и человека, потребляющего животные продукты. Использование радиоактивных изотопов позволяет проследить за поведением меченого инсектицида в животном организме и с большой точностью определить остаточное содержание инсектицидов и продуктов их распада в молоке и мясе.

В работе приводятся данные о скорости всасывания через кожный покров, накоплении в органах и тканях и выделении из организма лабораторных и сельскохозяйственных животных препарата ДДТ-C^{14}, полихлорпинена-Cl^{3} и хлорофоса-P^{32}.

EMPLEO DE LOS RADIOISÓTOPOS EN EL ESTUDIO DE LOS PROCESOS DE ABSORCIÓN, DISTRIBUCIÓN Y ELIMINACIÓN DE CIERTOS INSECTICIDAS EN EL ORGANISMO ANIMAL. El empleo de los insecticidas en la lucha contra los parásitos del ganado no debe perjudicar a la salud de éste ni de las personas que utilizan los productos animales. Los radioisótopos permiten seguir en el organismo animal el comportamiento de un insecticida marcado y determinar con gran precisión el contenido de residuos de insecticidas y de productos de su desintegración en la leche y en la carne.

En la memoria se presentan datos sobre la velocidad de absorción a través de la epidermis, la acumulación en los órganos y tejidos y la eliminación del DDT-^{14}C, del policlorpineno-^{36}Cl y del Clorosfos-^{32}P por el organismo de animales de laboratorio y por el del ganado.

Всесоюзный научно-исследовательский институт ветеринарной санитарии применяет радиоактивные изотопы для изучения процессов

всасывания, распределения и выделения из организма инсектицидов, используемых для борьбы с эктопаразитами сельскохозяйственных животных, а также при кожноводовой инвазии.

ИЗУЧЕНИЕ ВСАСЫВАНИЯ, ДЕПОНИРОВАНИЯ И ВЫДЕЛЕНИЯ ИЗ ОРГАНИЗМА ЖИВОТНЫХ ПРЕПАРАТА ДДТ, МЕЧЕННОГО C^{14}.

Широкое использование препаратов ДДТ (дихлордифенилтрихлорэтан) в ветеринарии как средства борьбы с эктопаразитами сельскохозяйственных животных и разноречивые данные о поведении его в организме сельскохозяйственных животных заставили провести специальное исследование по изучению вопросов всасывания, депонирования и выделения из организма животных этого препарата при нанесении его на кожу в виде масляного раствора в обычно применяемых дозах.

Меченный C^{14} препарат ДДТ в количестве 8,6 г растворялся в 200 мл вазелинового масла при подогревании. На одну обработку лактирующей коровы брали 100 мл раствора, в котором содержалось 4,3 г ДДТ с общей активностью 19,35 мккюри, что составляло 62 мккюри на 1 кг веса животного или 14 мг ДДТ на 1 кг живого веса.

Раствор втирался щеткой в область спины коровы от переднего края лопатки до корня хвоста. Активности препарата ДДТ, меченного C^{14}, в образцах крови, кала, мочи и молока определялись радиометрически на установке Б-2 с торцовым счетчиком Т-25-БФЛ. Полученные результаты переводились в весовые количества ДДТ.

В соответствии с ветеринарным законодательством корова обрабатывалась 4,3%-ным масляным раствором ДДТ дважды, с промежутком в 20 дней.

После первой обработки коровы уже через 2 часа в крови обнаруживается наличие ДДТ. Через сутки оно достигает 0,37 мг/л. Максимальное содержание ДДТ в крови - 0,43 мг/л обнаруживается к 6-му или 7-му дню, после чего резко уменьшается и с 10-го дня не обнаруживается.

После второй обработки (через 20 дней) ДДТ появляется в крови вновь и достигает максимальной величины на 13-й день (0,1 мг/л). Через 19 дней ДДТ в крови не обнаруживается.

Через 2 часа после первого нанесения на кожу 4,3%-ного масляного раствора ДДТ в пробах молока можно обнаружить C^{14}.

На следующий день в утреннем удое содержалось 0,48, а в вечернем - 2,78 мг/л ДДТ.

Максимальное количество C^{14} - ДДТ выделяется в молоке через 36 часов и соответствует 8,44 мг/л. Через двое суток содержание ДДТ в молоке уменьшается, однако до 10 - 12 суток содержание его превышает 1 мг/л. На 20-й день после первой обработки в молоке еще содержалось до 0,48 мг/л ДДТ.

После второй обработки (на 21 день) количество C^{14} - ДДТ в молоке возросло до 0,67 мг/л. Максимальное содержание ДДТ в молоке, после второй обработки, обнаружено на 4 сутки - 7,69 мг/л,

после чего идет постепенное уменьшение. Однако до 130 дней после обработки в молоке можно обнаружить C^{14} - ДДТ.

Всего за время проведения опыта с молоком выделилось около 590 мг ДДТ, что составляет 7% от количества, нанесенного на кожу.

За опытный период (130 дней) с мочой выделилось около 1149 мг, или 13,3%, а с калом - 1353 мг, или 15,7% от нанесенного количества ДДТ.

В органах и тканях подопытной коровы, забитой через 5 месяцев с момента первой обработки, обнаружено содержание ДДТ преимущественно в жировой ткани.

Так, в жире брыжейки, жире подкожной клетчатки и жире перикарда содержание ДДТ составляло 1,2 - 1,47 мг/кг.

Таким образом, за 5 месяцев после обработки коровы ДДТ выделяется около половины нанесенного препарата, причем наибольшее количество его выделяется в первые 4 суток.

Оставшийся в организме ДДТ депонируется во всех органах с преимуществом в жировой ткани.

ИЗУЧЕНИЕ ПРОНИЦАЕМОСТИ ЧЕРЕЗ КОЖУ, ДЕПОНИРОВАНИЯ И ВЫДЕЛЕНИЯ ИЗ ОРГАНИЗМА ЖИВОТНЫХ ПОЛИХЛОРПИНЕНА, МЕЧЕННОГО Cl^{36}

В опытах на кроликах и дойной корове изучалось всасывание, депонирование и выделение из организма препарата полихлорпинена, применяемого в стране для защиты животных от гнуса.

В опытах с нанесением 3%-ной эмульсии полихлорпинена, меченного Cl^{36} на кожу подопытных кроликов из расчета 0,75 мккюри (150 мг ПХП) на 1 кг живого веса, показали, что Cl^{36} появляется в крови через 1 час и обнаруживается в ней до 24 часов. В моче и кале Cl^{36} полихлорпинена появляется через 3 часа и выделяется с мочой в течение 96 часов, а с калом - 144 часов.

После забоя подопытных кроликов определяли содержание Cl^{36} в органах и тканях. Наибольшее количество его зарегистрировано в печени и почках.

В опытах на дойной корове с нанесением на кожу 3%-ной водной эмульсии меченого полихлорпинена в количестве 1,5 л с содержанием 225 мккюри Cl^{36} было установлено, что препарат ПХП быстро обнаруживается в крови и в небольших количествах регистрируется в течение 72 часов. В моче и кале Cl^{36} выделяется соответственно в течение 144 - 166 часов.

В молоке следы Cl^{36} обнаруживаются на 48 - 72 часе.

После четырехкратной обработки коровы эмульсией полихлорпинена Cl^{36} не обнаруживается в крови спустя 120 часов, а в моче и кале - после 168 часов.

Учитывая, что полихлорпинен не растворяется в воде при определении активности органов и тканей подопытных кроликов и коровы, измерение проводилось в водных и спиртовых вытяжках, при этом, как правило, Cl^{36} регистрировался только в водном экстракте. Отсюда было сделано предположение о быстром расщеплении молекулы ПХП в организме и выделения его в виде продуктов распада.

Для подтверждения этого был проведен хроматографический анализ ПХП и продуктов его распада, содержащихся в органах и тканях и выделяющихся с молоком, мочой и калом. Хроматография проводилась на бумаге Ленинградской фабрики марки "М" по видоизмененной методике Кишнера, Джона, Миллера и Келлера. В качестве подвижного растворителя применялся гексан и этилацетон.

Проявление хроматограмм производилось опрыскиванием бумажных полос 0,01%-ным водным раствором флюоресцеина и последующим выдерживанием хроматограмм в парах брома.

При этом флюоресцеин, реагируя с парами брома, дает красную окраску (образование эозина). В месте расположения полихлорпинена флюоресцеин сохраняет свою нормальную окраску, давая желтые пятна.

Данные хроматографического анализа подтвердили, что полихлорпинен в организме животного быстро расщепляется, так как ни в одном случае не было выделено пятен, Rf которых соответствовал бы цельной молекуле полихлорпинена.

ИЗУЧЕНИЕ МЕТАБОЛИЗМА ХЛОРОФОСА В ЖИВОТНОМ ОРГАНИЗМЕ

Хлорофос как фосфорорганический инсектицид в настоящее время широко применяется для обработки сельскохозяйственных животных против эктопаразитов и подкожного овода. В нашей лаборатории был синтезирован препарат метилового хлорофоса, меченный P^{32}, с удельной активностью 250 мккюри.

При наружной обработке лабораторных животных, а также крупного рогатого скота было установлено, что хлорофос в первые минуты после нанесения на кожу обнаруживается в крови, через 30 минут достигает максимальной величины, затем постепенно снижается и к 6 - 7-му дню не обнаруживается в крови.

Исследование проб молока дойной коровы показало, что через 30 минут в молоке появляются следовые количества P^{32} хлорофоса, через 3 - 6 часов активность образцов достигает максимальной величины, а затем уменьшается. На 6 - 7-й день радиоактивный фосфор хлорофоса в молоке обнаружить не удалось.

Выделение P^{32} хлорофоса с мочой достигает максимальной величины через 3 часа после нанесения на кожу и держится на высоком уровне до 6 часов. После 6 часов содержание P^{32} хлорофоса в моче снижается и на 3-й день становится в 6 раз меньше максимальной величины.

Однако и через 16 дней после обработки активность 1 мл мочи достигает 900 расп/мин. Выделение P^{32} хлорофоса с калом зарегистрировано через 2 часа после наружной обработки коровы меченым хлорофосом.

Максимальное количество P^{32} в кале обнаруживается через 48 часов, после чего уменьшается, и к 16 дню кал имеет небольшую активность.

Наряду с радиометрическим методом определения P^{32} пробы крови, мочи, кала, молока и внутренних органов кроликов и коровы подвергали хроматографическому анализу. Хроматография хлорофоса

и продуктов его распада проводилась на бумаге марки "Б" Ленинградской фабрики. В качестве подвижного растворителя использовалась смесь, состоящая из 2,5 частей 0,2%-го раствора соляной кислоты и 7,5 частей 95%-ного этилового спирта.

Проявление пятен осуществлялось опрыскиванием хроматограмм реактивом Бурроуса (25 мл 4%-ного раствора молибденовокислого аммония, 10 мл 1N раствора соляной кислоты, 5 мл 60%-ной хлорной кислоты и 60 мл дистиллированой воды).

После высушивания хроматограммы облучались кварцевой лампой в течение 2 - 5 минут. Соединения, содержащие фосфорные эфиры проявлялись в виде голубых пятен на белом фоне. Неорганические фосфаты обнаруживались в виде желтых пятен. В качестве свидетелей использовались препараты химически чистого хлорофоса, диметилфосфористой кислоты и ДДВФ, Rf которых соответственно равнялся: 0,75, 0,82 и 0,90.

Хроматография мочи и экстрактов печени подопытных животных, забитых в разные сроки после наружной обработки хлорофосом, показала отсутствие пятен, Rf которых соответствовал бы хлорофосу и ДДВФ. Обнаружены пятна, Rf которых соответствовал диметилфосфористой кислоте и неизвестным метаболитам. Значительная часть P^{32} содержалась в пятнах, соответствующих неорганическим фосфатам.

Не удалось также обнаружить хлорофос при хроматографическом исследовании молока коровы, обработанной хлорофосом.

В моче и экстрактах печени кроликов, которым хлорофос вводился per os, были обнаружены соединения, Rf которых соответствовал хлорофосу.

Изучение холинэстеразной активности крови, молока и внутренних органов подопытных животных подтвердило динамику всасывания и выведения из организма препарата хлорофоса.

DISCUSSION

R. von BORSTEL: What other insecticides are you planning to use in your future research?

G.V. FILATOV: Phosphoric organic compounds, mainly of the Trolene and Rogor types, and also carbamates of the Sevin type.

J. HALBERSTADT: Were any specific assay techniques used for determining the original compound and its break-down products in tissues, organs and excreta?

G.V. FILATOV: For this purpose we used the chromatographic method, and also inhibition of the choline esterase of standard serum.

STUDIES ON THE SELECTIVE TOXICITY OF SCHRADAN

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Abstract — Résumé — Аннотация — Resumen

STUDIES ON THE SELECTIVE TOXICITY OF SCHRADAN. Schradan is of low toxicity for chewing insects, while it is highly toxic to sucking insects. The selective toxicity of Schradan may be associated with distribution of toxicants in insect bodies, susceptibilities of insect cholinesterases to toxicants and metabolism of toxicants.

The absorption, excretion and metabolic rates of P^{32}-Schradan in those insects varied considerably and no definite relationships were found between those factors and toxicities, nor found between susceptibility of cholinesterases to toxicants and toxicities; but quantitative differences in P^{32}-Schradan distribution patterns existed among those insects. Much more P^{32}-Schradan accumulated in the central nervous systems of sucking insects than in those of chewing insects. Isolated nervous tissues of sucking insects absorbed much more P^{32}-Schradan than those of chewing insects.

Electron microscope observations on the central nervous sheath disclosed that the thoracic ganglia of chewing insects were enclosed in a thick and robust sheath, while those of sucking insects were surrounded by simple, thin double membranes.

The author came to the conclusion that the selective toxicity of Schradan to insects was not due to the difference of their metabolizing activities of Schradan as regards activation and detoxication, nor to the sensitivity of insect cholinesterases to activated Schradan, but to the differences of Schradan distribution patterns in insect bodies. Thus the distribution of toxicants in insects and the character of the nerve sheath, which acts as a barrier against the penetration of toxicants, may be the most important factors responsible for the selective toxicity of Schradan.

ÉTUDE DE LA TOXICITÉ SÉLECTIVE DU SCHRADAN. Le Schradan est peu toxique pour les insectes broyeurs, alors qu'il l'est extrêmement pour les insectes suceurs. Sa toxicité sélective pourrait dépendre de la distribution des substances toxiques dans l'organisme de l'insecte, de leur action sur la cholinestérase et de leur métabolisme.

On a observé des différences considérables, selon les insectes, dans les taux d'absorption et d'excrétion et dans le métabolisme du Schradan marqué par ^{32}P, mais on n'a pas pu établir de rapport bien défini entre ces facteurs et la toxicité, pas plus qu'entre la sensibilité de la cholinestérase aux substances toxiques et leur toxicité. Par contre, on a relevé des différences quantitatives dans la distribution du Schradan-^{32}P. L'accumulation de Schradan-^{32}P a été beaucoup plus forte dans le système nerveux central des insectes suceurs que dans celui des insectes broyeurs. De même, les tissus nerveux isolés des premiers ont absorbé beaucoup plus de Schradan-^{32}P que ceux des insectes broyeurs.

L'observation au microscope électronique des gaines dans les centres nerveux a révélé que les ganglions thoraciques des insectes broyeurs sont revêtus d'une gaine épaisse et robuste, tandis que ceux des insectes suceurs sont entourés d'une membrane simple ou d'une membrane mince et double.

L'auteur est parvenu à la conclusion que la toxicité sélective du Schradan pour les insectes ne tient pas aux différences de métabolisme du Schradan au cours des phases d'activation et de détoxication, ni à la sensibilité de la cholinestérase au Schradan marqué, mais qu'elle est due aux différences de distribution du Schradan dans l'organisme des insectes. La distribution des substances toxiques dans l'organisme des insectes et la nature de la gaine des nerfs, qui s'oppose à la pénétration des substances toxiques, pourraient donc constituer les principaux facteurs de la toxicité sélective du Schradan.

ИЗУЧЕНИЕ СЕЛЕКТИВНОЙ ТОКСИЧНОСТИ ШРАДАНА. Шредан мало токсичен для жующих, но высоко токсичен для сосущих насекомых. Селективная токсичность шредана может быть связана с распределением токсических веществ в теле насекомых, с чувствительностью холинэстеразы насекомых к токсическим веществам и с метаболизмом последних.

Поглощение, выделение и скорости метаболизма прадана, меченного фосфором-32, значительно различались у этих насекомых, и определенных связей между этими факторами и токсичностью установлено не было, так же как не было установлено определенных связей между чувствительностью холинэстеразы к токсическим веществам и к токсичности. Однако у этих насекомых наблюдались количественные различия в распределении прадана, меченного фосфором-32. У сосущих насекомых в центральной нервной системе сосредоточивалось гораздо больше меченного фосфором-32 прадана, чем у жующих насекомых. Изолированные нервные ткани сосущих насекомых поглощали гораздо больше прадана, меченного фосфором-32, чем нервные ткани жующих насекомых.

Наблюдения под электронным микроскопом оболочки центральной нервной системы показали, что грудные ганглии жующих насекомых были покрыты толстой и прочной оболочкой, тогда как те же органы сосущих насекомых были окружены простой и тонкой двойной мембраной.

Авторы пришли к заключению, что селективная токсичность прадана для насекомых объясняется не разницей в метаболических процессах активации и детоксикации прадана и не чувствительностью холинэстеразы насекомых к активированному прадану, а разницей в распределении его в теле насекомых. Таким образом, распределение токсических веществ в теле насекомых и характер оболочки нервной системы, являющейся барьером для проникновения токсических веществ, являются, по-видимому, наиболее важными факторами, объединяющими селективную токсичность прадана.

ESTUDIO DE LA TOXICIDAD SELECTIVA DEL SCHRADAN. El Schradan es poco tóxico para los insectos masticadores mientras que lo es en alto grado para los chupadores. Su toxicidad selectiva quizá esté asociada a la distribución de los tóxicos en el organismo de los insectos, a la sensibilidad de las colinesterasas de los insectos a los tóxicos, y al metabolismo de éstos.

Las velocidades de absorción, excreción y metabolismo del Schradan-^{32}P varían mucho en estos dos tipos de insectos y no se pudo encontrar ninguna relación clara entre estos factores y la toxicidad, ni entre la sensibilidad de las colinesterasas a los tóxicos y la toxicidad. En cambio se registraron diferencias cuantitativas entre los esquemas de distribución del Schradan-^{32}P en estos dos tipos de insectos. Se acumula mucho más Schradan-^{32}P en el sistema nervioso central de los insectos chupadores que en el de los masticadores. Los tejidos nerviosos aislados de los insectos chupadores absorben mucho más Schradan-^{32}P que los de los insectos masticadores.

Observando con el microscopio electrónico la vaina del sistema nervioso central se pudo comprobar que los ganglios torácicos de los insectos masticadores poseen una vaina gruesa y robusta, mientras que los de los insectos chupadores están rodeados de una sola membrana o de membranas dobles muy delgadas.

El autor llegó a la conclusión de que la toxicidad selectiva del Schradan para estos insectos no se debe a la diferencia que hay entre la actividad metabólica del Schradan en la activación o la desintoxicación ni a la sensibilidad de las colinesterasas del insecto al Schradan activado, sino al diferente esquema de distribución del insecticida en el organismo del insecto. Así, pues, los factores más importantes que determinan la toxicidad selectiva del Schradan pueden ser la distribución del tóxico en los insectos y el carácter de la vaina nerviosa que hace de barrera contra su penetración.

INTRODUCTION

Generally, a given insecticide is not always equally effective against all kinds of insects. Systemic insecticides, which are absorbed by a plant and translocated to other parts of the plant, so that insecticidal activity is transferred to untreated parts, are effective against sucking insects, but not so effective against chewing insects.

Schradan, which is one of the systemic insecticides, was found to be of low toxicity for the larva of the rice stem-borer, Chilo suppressalis Walker, the adult of the American cockroach, Periplaneta americana L., and the adult of the house-fly, Musca domestica vicina Macq., while it was highly toxic for the adult of the rice bug, Leptocorisa varicornis Fabricius, the black rice bug, Scotinophara lurida Burmeister, and the green rice leafhopper, Nephotettix bipunctatus cincticeps Uhler (Table I).

TABLE I

TOXICITIES OF SCHRADAN APPLIED TOPICALLY TO VARIOUS INSECTS

Insect	LD50 (μg/g)	Regression coefficient
Rice stem-borer	>26 222	--
American cockroach	2 170	0.251
House-fly	1 932	0.242
Green rice leafhopper	160	0.649
Black rice bug	92	0.394
Rice bug	23>	-

Schradan is converted, by enzymatic oxidation in the insect body, into oxidized Schradan, a strong anticholinesterase. The inhibition of cholinesterase in the central nervous system may be the principal action of Schradan. Consequently the selective toxicity of Schradan may be associated with the following three main factors:

(1) Differences in the distribution of toxicants in insect bodies;
(2) Differences in the susceptibility of insect cholinesterase to toxicants; and
(3) Differences in the metabolism of toxicants as regards activation and detoxification.

TABLE II

ABSORPTION AND EXCRETION OF SCHRADAN BY VARIOUS INSECTS AFTER TOPICAL APPLICATION (36 μg Schradan per insect)

Insect	Absorption/excretion (%)					
	After 1 h			After 6 h		
	Outer	Internal	Excreta	Outer	Internal	Excreta
Rice stem-borer	26.5	3.7	69.8	11.4	12.1	76.5
American cockroach	31.1	27.7	41.2	8.8	42.8	48.4
House-fly	11.5	1.8	86.7	8.2	3.0	88.8
Green rice leafhopper	20.6	3.3	76.1	18.5	4.3	77.2
Black rice bug	17.8	18.7	63.5	13.4	10.4	76.2
Rice bug	19.6	3.1	77.3	11.8	11.3	76.9

RESULTS

(a) Distribution of Schradan in various insect species

No quantitative differences concerning the absorption and excretion capacities of Schradan were observed as between the susceptible and non-susceptible insects (Table II). There were, however, quantitative differences in the Schradan distribution patterns. Much more Schradan accumulated in the central nervous systems of susceptible insects than in those of non-susceptible insects (Tables III, IV).

(b) Cholinesterase inhibition of Schradan in various insect species

Schradan itself is a very weak anticholinesterase, but oxidized Schradan is a powerful anticholinesterase, oxidation enhancing the activity by as much as 1000-10 000 times. The susceptibility of the cholinesterases of the various insects to Schradan and to oxidized Schradan varied considerably, and no definite relationships were found between enzyme inhibition and toxicity (Table V).

(c) Metabolism of Schradan in various insect species

Schradan was activated and detoxicated by the species studied. The metabolic rates of Schradan in those species varied considerably and no definite relationships were found between metabolism and toxicity (Table VI).

(d) Penetration of Schradan and oxidized Schradan to isolated nervous tissues in various insect species

Isolated nervous tissues of susceptible insects absorbed much more Schradan than those of non-susceptible insects (Table VII), and desheathed nervous tissues of cockroach absorbed much more Schradan and oxidized Schradan than untreated tissues (Table VIII).

(e) Electron-microscope observation of the ganglionic sheaths of various insect species

Electron-microscope studies on the central nervous sheath showed that the thoracic ganglia of non-susceptible insects were enclosed in a thick and robust sheath, while those of susceptible insects were surrounded by single or thin double membranes.

DISCUSSION

Several hypotheses have been put forward to explain the physiological selectivity of Schradan. CASIDA et al. have shown that the susceptibility of insects to Schradan depends upon the sensitivity of the particular insect cholinesterase for the activated Schradan and that the cholinesterase from the non-susceptible insects required 6-24 times as much activated Schradan

TABLE III

DISTRIBUTION OF SCHRADAN IN THE TISSUES OF VARIOUS INSECTS TREATED WITH 500 $\mu g/g$ P^{32}-SCHRADAN BY TOPICAL APPLICATION
(Percentages)

Insect	Nerve cord	Thoracic ganglion	Gut	Fat	Coxa	Reproductive system	Remainder
Rice stem-borer	0.008	-	0.202	0.490	-	-	0.451
American cockroach	0.151	-	3.146	1.113	1.971	0.147	10.623
House-fly	-	0.053	0.403	0.090	0.105	0.158	2.153
Green rice leafhopper	-	0.583	0.583	0.292	-	-	16.917
Black rice bug	-	0.600	1.400	0.500	0.900	0.300	22.600
Rice bug	-	0.500	2.288	1.500	2.250	0.875	25.119

TABLE IV

DISTRIBUTION OF SCHRADAN IN THE TISSUES OF VARIOUS INSECTS TREATED WITH 500 μg/g P^{32}-SCHRADAN BY TOPICAL APPLICATION
(Amounts, μg/mg)

Insect	Nerve cord	Thoracic ganglion	Gut	Fat	Coxa	Reproductive system	Remainder
Rice stem-borer	0.019	-	0.011	0.008	-	-	0.003
American cockroach	0.068	-	0.135	0.058	0.055	0.044	0.073
House-fly	-	0.025	0.007	0.007	0.017	0.008	0.007
Green rice leafhopper	-	0.100	0.013	0.023	-	-	0.024
Black rice bug	-	1.500	0.046	0.085	0.188	0.142	0.121
Rice bug	-	0.500	0.087	0.100	0.300	0.350	0.095

TABLE V

MOLAR CONCENTRATIONS OF SCHRADAN AND OXIDIZED SCHRADAN FOR 50% AND 80% CHOLINESTERASE INHIBITIONS (mM)

Insect	Schradan		Oxidized Schradan	
	Inhibition		Inhibition	
	50%	80%	50%	80%
Rice stem-borer	800	2700	1.0	2.2
American cockroach	1200	2300	4.2	6.8
House-fly	40	380	0.0098	0.025
Green rice leafhopper	200	720	0.98	3.0
Black rice bug	410	1300	2.4	5.6
Rice bug	1100	2800	3.2	5.8

for inhibition as that of the susceptible insects [1,2]. O'BRIEN and SPENCER [3, 4, 5], on the other hand, considered that the physiological selectivity of Schradan was not due to the sensitivity of the particular insect cholinesterase to the Schradan metabolite, but that susceptible insects converted Schradan to the cholinergic oxidation product slowly within the nerve tissue, while in the non-susceptible species the conversion rate in the fat body of the insect was so great that little or no unconverted Schradan reached the nerve tissue and the oxidation product of Schradan was too unstable to penetrate the lipoid nerve sheath. They proposed the hypothesis that non-susceptible insects have a barrier which prevents the penetration of the converted Schradan to the site of action. TSUYUKI et al. suggested [6] that the active anti-cholinesterase agent was isomerized to a much more stable component, the methyl ether. They considered that the selective toxicity of Schradan might be associated with this isomerization.

CONCLUSION

The author came to the conclusion that the selective toxicity of Schradan to insects was not due to differences in their metabolism of Schradan as regards activation and detoxication, nor to the sensitivity of insect cholinesterases to activated Schradan, but to the differences of Schradan distribution patterns in insect bodies. Thus the distribution of toxicants in insects and the nature of the nerve sheath which acts as a barrier against the penetration of toxicants may be the most important factors responsible for the selective toxicity of Schradan.

TABLE VI

PER-CENT METABOLISM OF P^{32}-SCHRADAN INDICATED BY SLICE OF TISSUE IN VARIOUS INSECT SPECIES (30°C, 3 h)

Insect					Corrected	
	Schradan	Oxidized Schradan	Hydrozates	Protein incorporates	Hydrozates	Oxidized Schradan
Rice stem-borer	94.8	1.2	3.8	0.2	3.6	1.6
American cockroach	90.2	1.0	7.9	0.9	7.0	2.8
House-fly	93.4	1.7	4.5	0.4	4.2	2.4
Green rice leafhopper	92.6	1.2	5.6	0.6	5.0	2.4
Rice bug	97.0	0.8	1.8	0.2	1.7	1.3

TABLE VII

ABSORPTION OF P^{32}-SCHRADAN BY ISOLATED NERVE TISSUES OF VARIOUS INSECTS IMMERSED IN PHOSPHATE-BUFFER RINGER SOLUTION OF 0.1% SCHRADAN (pH 7.1, 30°C, after 60 min)

Nerve tissues	Tissue weight (mg)	Absorbed Schradan (μg/mg)
Rice stem-borer	0.16	0.313
American cockroach	11.20	0.528
House-fly	0.16	0.506
Green rice leafhopper	0.06	0.583
Black rice bug	0.10	0.770
Rice bug	0.14	0.621
Desheathed American cockroach	9.90	0.612

TABLE VIII

ABSORPTION OF SCHRADAN AND OXIDIZED SCHRADAN BY VENTRAL NERVE CORD OF THE AMERICAN COCKROACH

Insecticides	Schradan in Ringer solution					Oxidized Schradan
Concentrations (%)		0.1%		0.0114	0.0006	0.0114
Immersion times (min)	10	20	40	40	40	40
Absorption in normal nerve cord (μg/mg)	0.195	0.249	0.303	0.074	0.001	0.079
Absorption in desheathed nerve cord (μg/mg)	0.289	0.275	0.317	0.118	0.003	0.104

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DISCUSSION

S. FUZEAU-BRAESCH: Do you possess strains resistant to this insecticide, and if so can you compare insects of the same species, susceptible or non-susceptible, instead of comparing different species?

T. SAITO: The study reported compares species which were naturally susceptible or resistant to Schradan. No strains of any of the susceptible species which had acquired resistance through treatment with this compound were available for study.

J.E. CASIDA: How did you prepare oxidized Schradan?

T. SAITO: The oxidized P^{32}-Schradan was prepared by potassium permanganate oxidation from P^{32}-Schradan and separated by silica-gel chromatography, by the method described in ref. [6] to the paper. One fraction was then analysed by means of infra-red spectra, paper chromatography, anticholinesterase activity and chloroform-water partition coefficient.

K. van ASPEREN: I have an idea that O'Brien and Spencer suggested that resistant insects may oxidize the Schradan outside the nervous system so quickly that very little penetration of the now more polar substance through the nerve sheath could occur. If that is true, their explanation of differences in toxicity in different insects is more or less similar to yours, i.e. a difference in distribution. However, my memory may mislead me.

T. SAITO: I had some correspondence with O'Brien on this problem. I think that the different results may be due to the different methods. O'Brien used enzymatic analysis, but I am analysing radiometrically in situ.

C.C. HASSETT (Chairman): I might make a general comment on the problem of the insect nerve sheath in relation to Dr. Saito's work. This sheath is a relatively impermeable barrier to drugs and it was not until Roeder and colleagues devised the technique of removal that it was found that the insect nervous system responded to various agents in much the same way as the mammalian system.

J.E. CASIDA: Perhaps I could also add a comment. Dr. Saito is right in saying that O'Brien and Spencer postulated that the nerve sheath was a barrier in the resistant species more than in the susceptible species. However, it is easy for the author of a paper to throw out several hypotheses more or less in passing; as you know, the more one works with how a compound acts on an insect in relation to resistance, be it natural or acquired, the more one comes to the conclusion that the effects cannot be pinned down to a single factor. I think the evidence Dr. Saito has presented for the role of the nerve sheath shows better than anything else that this is a major contributing factor.

D. F. HEATH: Your results undoubtedly indicate that differences in distribution are important, but taking the results as a whole one would conclude that the house-fly should be the most susceptible species, since house-fly anticholinesterase was found to be inhibited at very much lower concentrations than the anticholinesterase of any other species, and this would quite outweigh the other effects you observed.

This does not necessarily mean that your conclusions are wrong. The concentration of inhibitor in the nervous system as a whole can only be a very crude indicator of the concentration at the synapses. De-sheathing a locust ganglion preparation increases the sensitivity of the preparation to eserine and acetylcholine, but the ganglion is still much less sensitive than mammalian ganglia. This and other evidence suggests that the synapses are protected by additional membranes. Consequently, the concentration of inhibitor in the synaptic region cannot be assumed to be similar to the concentration in a whole nerve. It would be very useful to determine inhibitor concentrations in the synaptic regions, but I have no idea of how this could be done.

IV.

INSECT METABOLISM: TRACER APPLICATIONS

STUDIES ON THE UTILIZATION, METABOLISM AND FUNCTION OF STEROLS IN THE HOUSE-FLY, MUSCA DOMESTICA

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Abstract — Résumé — Аннотация — Resumen

STUDIES ON THE UTILIZATION, METABOLISM AND FUNCTION OF STEROLS IN THE HOUSE-FLY, MUSCA DOMESTICA. Insects generally have been found to require a dietary source of sterol for normal larval growth and metamorphosis. Our work has pointed to two additional physiological roles for sterols in the house-fly, Musca domestica L.: (1) A dietary source of sterol is essential for sustained viable egg production in the female fly; on a sterol-deficient diet eggs are produced but hatch and viability are low. (2) Cholesterol is also involved in the mobilization and utilization of nutrient reserves associated with the initiation of ovarian maturation in the female fly.

The quantitative sterol requirements for the above physiological processes and the metabolic conversions that occur during growth, metamorphosis and reproduction have been studied in this insect, using C^{14}- and H^3-labelled sterols in conjunction with a variety of analytical tools, including reverse isotope dilution, gas-liquid chromatography and spectroscopy, and employing aseptic rearing techniques and semi-defined larval and adult diets.

Both C^{14}-cholesterol and H^3-β-sitosterol have been used as a source of sterol in either the larval or the adult diet of the house fly, and the pattern of utilization and metabolism was found to be almost identical for these two sterols. However, there was no detectable conversion of β-sitosterol to cholesterol.

Sub-minimal quantities of cholesterol have also been used in the larval diet in combination with "sparing sterols" such as cholestanol, which will fulfill in part but not entirely the sterol requirement of this insect. The utilization and fate of the "sparing sterol" has been investigated using C^{14}-cholestanol, and the metabolism of the minute quantity of essential cholesterol is currently under study using high-specific-activity C^{14}-cholesterol.

Other species of insects, including the German cockroach (Blattella germanica), have been examined in relation to the patterns of utilization and the metabolic pathways for sterols found in the house-fly.

UTILISATION, MÉTABOLISME ET FONCTIONS DES STÉROLS CHEZ LA MOUCHE DOMESTIQUE (MUSCA DOMESTICA). On a déterminé que, de façon générale, les insectes ont besoin de stérols dans leur régime alimentaire pour que leurs larves se développent et se métamorphosent normalement. Les travaux de l'auteur ont fait apparaître deux autres fonctions physiologiques des stérols chez la mouche domestique (Musca domestica L.): 1. il est indispensable que le régime alimentaire de la femelle contienne des stérols pour que celle-ci ponde régulièrement des œufs viables; s'il y a carence de stérols, la femelle continue à pondre mais seul un faible pourcentage des œufs parviennent à éclosion et sont viables; 2. le cholestérol joue également un rôle dans l'utilisation des réserves nutritives dont s'accompagne le début du développement des ovaires.

On a étudié chez cet insecte les quantités de stérols qui sont nécessaires pour que les processus physiologiques ci-dessus se déroulent, ainsi que les transformations métaboliques qui se produisent au cours du développement, de la métamorphose et de la reproduction. Cette étude a été faite à l'aide de stérols marqués par ^{14}C et ^3H, en appliquant diverses méthodes analytiques: dilution isotopique inversée, chromatographie à phase gazeuse et phase liquide, spectroscopie; on a eu recours à des méthodes d'élevage en milieu aseptique et on a établi des régimes alimentaires semi-contrôlés pour les larves et les adultes.

Le cholestérol marqué par ^{14}C et le sitostérol-β marqué par ^3H ont été utilisés comme sources de stérols dans le régime alimentaire de la mouche domestique aux stades larvaire et adulte; il s'est révélé que l'utilisation et le métabolisme de ces deux stérols étaient à peu près identiques. Toutefois, on n'a pas observé de transformation du sitostérol-β en cholestérol.

Dans le régime alimentaire des larves on a également fait entrer du cholestérol en quantité inférieure aux besoins minima et on y a ajouté des «stérols pauvres» comme le cholestanol, qui ne satisfont que partiellement les besoins en stérols de l'insecte. L'utilisation et le sort du «stérol pauvre» ont été étudiés à l'aide de cholestanol marqué par ^{14}C; à l'aide de cholestérol marqué par ^{14}C, d'activité spécifique élevée, on poursuit les recherches sur le métabolisme de la microquantité de cholestérol indispensable.

On a étudié chez d'autres espèces d'insectes, notamment la blatte allemande (Blattela germanica), l'utilisation et les voies métaboliques des stérols et on les a comparées à celles qui ont été observées chez la mouche domestique.

ИЗУЧЕНИЕ УСВОЕНИЯ, МЕТАБОЛИЗМА И ФУНКЦИИ СТЕРИНОВ В ОРГАНИЗМЕ ДОМАШНЕЙ МУХИ MUSCA DOMESTICA. Было обнаружено, что большинству насекомых нужен диетический источник стеринов для нормального роста и метаморфоза личинки. Работа посвящена двум дополнительным физиологическим функциям стеринов в организме домашней мухи Musca domestica L.: 1) Источник стеринового питания совершенно необходим для образования выносливых жизнеспособных яиц у женских особей мухи; при недостаточном стериновом питании яйца образуются, но их выносливость и жизнеспособность являются низкими. 2) Холестерин также играет роль в мобилизации и использовании резервов питания при созревании яичников у самок домашних мух.

Изучалась количественная потребность в стерине при вышеуказанных физиологических процессах и метаболических превращениях, происходящих во время роста, метаморфоза и размножения у этих насекомых. Исследования проводились с помощью меченных углеродом-14 и тритием стеринов в сочетании с различными аналитическими методами, включая обратное изотопное разбавление, газово-жидкостную хроматографию, спектроскопию, и с помощью методов асептического культивирования и пищевых режимов для полуоформировавшихся личинок и взрослых особей.

Холестерин, меченный C^{14}, и β-ситостерин, меченный тритием, использовались в качестве источника стерина в рационе или личинок, или взрослых особей домашней мухи. Было обнаружено, что характер усвоения и метаболизм этих двух стеринов почти одинаковы. Однако не было поддающегося обнаружению превращения меченного тритием β-ситостерина в холестерин.

В рационе личинки использовались также субминимальные количества холестерина в сочетании с "резервными стеринами", например с холестанолом, который удовлетворял частично, но не полностью потребности этого насекомого в стерине. Усвоение и судьба "резервного стерина" исследовались при помощи холестанола, меченного углеродом-14, а в настоящее время с помощью холестерина, меченного углеродом-14, с высокой удельной активностью, изучается метаболизм малых количеств исходного холестерина.

ESTUDIOS SOBRE LA ASIMILACIÓN, EL METABOLISMO Y LA FUNCIÓN DE LOS ESTEROLES EN LA MOSCA COMÚN (MUSCA DOMESTICA). Se ha descubierto que en general los insectos necesitan consumir habitualmente esterol para que el desarrollo de las larvas y las metamorfosis se efectúen en condiciones normales. El presente trabajo se refiere a otras dos funciones fisiológicas del esterol en la mosca común (Musca domestica L.), que pueden concretarse del modo siguiente: 1) Las hembras necesitan consumir habitualmente esterol para poder producir huevos fértiles; en caso de consumo insuficiente, los huevos son de escasa fertilidad y viabilidad; 2) El colesterol interviene también en el mecanismo de utilización de las reservas nutritivas en la mosca hembra cuando se inicia la maduración ovárica.

La cantidad de esterol necesaria para el proceso fisiológico indicado y las transformaciones metabólicas que se producen durante el desarrollo, la metamorfosis y la reproducción han sido estudiadas en este insecto empleando esteroles marcados con ^{14}C y ^{3}H; al mismo tiempo se ha hecho uso de diversos procedimientos de análisis, entre ellos la dilución isotópica recíproca, la cromatografía gas-líquido y la espectroscopía, y se han utilizado procedimientos de cría en medio aséptico y dietas semidefinidas de larvas y adultos.

Como fuente de esterol en la dieta de los individuos adultos o de las larvas de mosca común, se emplearon el colesterol-^{14}C y el sitosterol-β-^{3}H, habiendo sido casi idénticos en ambos casos el proceso de asimilación y metabolismo de estos esteroles. Sin embargo, no hubo transformación apreciable de β-sitosterol en colesterol.

En las dietas de las larvas se utilizaron también cantidades de colesterol inferiores al mínimo necesario, combinadas con «esteroles de ahorro», como el colestanol, que pueden suministrar sólo una parte del esterol que necesita el insecto. La asimilación y el destino final del «esterol de ahorro» se han estudiado empleando colestanol-^{14}C; también se está estudiando el metabolismo de la minúscula cantidad de colesterol esencial, empleando para ello colesterol-^{14}C de elevada actividad específica.

Se han utilizado también otras especies de insectos, entre ellos la cucaracha alemana (Blattella germanica), para completar el estudio de los modos de asimilación y los esquemas metabólicos de los esteroles en la mosca común.

INTRODUCTION

In 1935, HOBSON [1] reported that larvae of the blowfly, Phaenicia sericata, require a dietary source of sterol for normal growth and development. Since this discovery, the immature stages of a number of insects, representing several of the major orders, have been examined as to their sterol requirement and every insect studied has been found to require a dietary or exogenous source of sterol for normal larval growth and metamorphosis [2]. This nutritional requirement for sterols has generally been considered to indicate the absence of sterol biosynthesis in insects and this has been confirmed biochemically in several species, using C^{14}-labelled sterol precursors [3-10]. However, there is some indication that certain of the more primitive insects may be capable of some degree of cholesterol biosynthesis [11, 12].

Although cholesterol has generally been used as the dietary sterol and has been found to support growth in every species studied, many omnivorous and phytophagous insects can also use certain C-28 and C-29 phytosterols in lieu of cholesterol. For certain carnivorous insects, such as the hide beetle, Dermestes maculatus, however, only cholesterol and certain closely related C-27 sterols are satisfactory sterol sources [13, 4]. Using this insect, CLARK et al. [14], in a particularly significant study, recently provided us with a working hypothesis for investigating both the fate and function(s) of sterols in insects. These workers have shown that the hide beetle will grow and mature on a larval diet containing sub-minimal quantities of cholesterol if the diet is supplemented by certain "sparing-sterols" which alone will not fulfil the sterol requirement of this insect. Based on their work, they have proposed a dual role for sterols in insects. They postulate that the "sparing-sterols", which will replace up to 97% of the cholesterol requirement of Dermestes, serve in a structural capacity, as cellular or subcellular components. The remaining irreplaceable cholesterol is believed to serve a metabolic role, perhaps as a precursor for physiologically active sterol metabolites. However, in a subsequent study on the metabolism of C^{14}-cholesterol by the cockroach, Eurycotis floridana, under "sparing" conditions, CLAYTON et al. [15] found that the minimum essential cholesterol requirements of this insect were not completely metabolized and suggested that in insects cholesterol may also have a specific structural role which cannot be fulfilled by the "sparing-sterols".

In addition to larval growth and metamorphosis, sterols have been reported to play a role in several other physiological processes. Certain phytosterols appear to act as phagostimulants for silkworm larvae, Bombyx mori, [16, 17] and a dietary source of sterol has been reported to be essential for normal reproduction in the German cockroach, Blattella germanica [18]. In the house fly, Musca domestica, we have found sterols to be involved in the initiation of ovarian development [19] and to be necessary for sustained viable egg production [20, 21]. Finally, the recent reports by KOBAYASHI et al. [22, 23] that cholesterol has "brain hormone" activity

in debrained, diapausing silkworm pupae, suggest that in insects sterols may either serve as hormones per se or as precursors to hormones.

From this brief review it is apparent that sterols are either involved in or essential to a number of the important physiological and biochemical processes of insects.

Information on the biochemical and physiological function(s) of sterols and their metabolic fate in insects is not only important for a more complete knowledge of insect physiology and biochemistry, but should also add to our general understanding of the role(s) of neutral sterols in living systems generally.

Both the minute quantities of cholesterol required, especially when used in combination with other sterols, and the small size of many insects, make it difficult to follow the uptake, utilization, metabolism, and transfer of cholesterol from one development stage to another. Radioisotope-labelled sterols, which have been extensively used in this area of research, have not only provided an extremely sensitive and efficient technique for following the above processes, but have permitted certain experiments to be carried out with ease which otherwise would have been extremely difficult, if not impossible.

The purpose of this paper is to briefly report on certain facets of our research on the function(s) and metabolic fate of sterols in the house fly, Musca domestica, and in so doing to illustrate some applications of radioactive-tracer techniques to this area of insect physiology.

MATERIALS AND METHODS

The house flies used in these studies were from a 1948 NAIDM strain. The stock colonies were reared by the CSMA procedure [24] and fed an adult diet of dry defatted milk-sucrose (1:1). Unless otherwise noted, a semi-defined adult diet [21] and an aseptic semi-defined larval diet [25] were employed in these experiments. The "vitamin-free" casein used in preparing these diets was exhaustively extracted to remove sterols. The C^{14}- and H^3-labelled sterols were repurified just before use and their radiochemical purity checked by several analytical methods. All radioassays were made on replicated "weightless" samples using windowless gas-flow proportional counters. Gas-liquid chromatographic analyses of the sterols were made using the systems of VANDENHEUVEL et al. [26].

STEROLS AND REPRODUCTION

In contrast to the numerous studies on the requirement for sterols by immature insects, our knowledge of the role of sterols in the adult insect is extremely limited. MONROE [20, 21], in a study from our laboratory, has shown that flies fed on a sterol-deficient semi-defined diet lived as long and produced approximately the same number of eggs as flies fed on the same diet containing cholesterol. However, the cholesterol deficiency caused an overall reduction of about 80% in egg hatch or viable egg production. When flies were fed on the semi-defined diet containing cholesterol, egg hatch ranged from 82 to 98% throughout all the egg collections. When cholesterol

was omitted from the diet, egg hatch was initially lower and decreased rapidly until by the fourth egg collection it was only 5%. If at this time these flies were given a diet containing cholesterol, the egg hatch was found to increase progressively until by the ninth collection it was similar to that found for the eggs of flies fed the diet plus cholesterol. In addition, only about 50% of the larvae from the eggs from flies fed on the sterol-deficient diet produced adults as compared with 91% of those from the diet containing cholesterol.

The production of some viable eggs up to about the fourth egg collection suggested a physiological mechanism whereby the female fly may use sterols stored from the larval diet for the early groups of eggs produced. The presence of such a storage mechanism was confirmed in a subsequent study [27].

The above-mentioned finding prompted us to examine the relative contribution of the stored sterols and the sterols from the adult diet to the total sterol content of the eggs. This was accomplished by using either C^{14}-labelled or unlabelled cholesterol in the larval diet and C^{14}-cholesterol in the adult diets and then examining the first groups of eggs produced for both the sterol content and the specific activity of the sterols present. Under our rearing conditions, and using optimal concentrations of cholesterol in both the larval and adult diets, we have found that as much as 75% of the sterol present in the first group of eggs produced originates from the stored reserves of the female fly. However, when flies are reared on larval diets containing combinations of "sparing sterols" and sub-minimal quantities of cholesterol, the adult females, which contain very little, if any, excess cholesterol, then must depend almost wholly on the sterols from the adult diet for egg production [28].

STEROLS OF HOUSE-FLY EGGS

From the above studies, it is apparent that the sterols present in the house-fly egg are essential for both normal embryonic development and early larval growth. These findings have prompted us to undertake a thorough study on both the amount and nature of the sterols present in the eggs and to examine any qualitative or quantitative changes that may occur in the sterols during these early developmental stages.

An earlier study [29], in which we injected C^{14}-cholesterol into adult female flies reared by the CSMA procedure, provided us with some qualitative information on the egg sterols. The injected C^{14}-cholesterol was efficiently incorporated into the eggs where it was found to be present in both the free and esterified form. A portion of the C^{14}-cholesterol in the egg and adult fly had been converted to its 5,7-diene, 7-dehydro-cholesterol. However, because of the presence in these eggs of phytosterols from the adult flies and unlabelled cholesterol from the adult diet, the total sterol content could not be studied.

Eggs from flies reared aseptically on a semi-defined larval diet containing 0.2% C^{14}-cholesterol and fed on a semi-defined adult diet containing 0.1% C^{14}-cholesterol were used for the quantitative studies. These eggs were found to contain about 0.051 μg-equivalents of radioactive sterol per egg. When the crude total lipid from these eggs was fractionated into lipid classes by column chromatography on silicic acid, two major radioactive peaks, representing sterol esters and free sterols, were detected [30]. In

these eggs the sterol esters accounted for about 41% of the total C^{14}-sterols present, but in some of our other studies we have found ester fractions as high as 60%. Analysis by ultra-violet (UV) spectroscopy of either the total lipids before fractionation or of the free sterol and sterol ester fractions indicated the presence of an appreciable amount of a 5,7-diene. For confirmation of this finding, the total sterols, as their acetates, were fractionated by chromatography on an alumina column which separates Δ^5 and $\Delta^{5,7}$ sterols. Two major radioactive peaks were detected: The major peak (Δ^5 fraction), which accounted for about 86% of the radioactive material, was identified as cholesterol by reverse-isotope dilution and purification through the dibromide and by its relative retention time in several gas-liquid chromatographic systems. The minor, more polar, peak ($\Delta^{5,7}$ fraction) contained about 13.8% of the total radioactivity and, when analysed by UV spectroscopy, was found to have a spectrum and molecular extinction coefficient, computed from the micromoles of radioactive sterol present, identical to 7-dehydrocholesterol [28]. Further confirmation of the identity of the compound has been made using gas-liquid chromatography and reverse-isotope dilution. A summary of our findings on the sterol content of house-fly eggs is presented in Table I. Although we have not determined the minimum sterol requirement for egg viability, we have found that as little as 0.015 μg total sterol per egg, or 25 to 30% of the maximum sterol content we have observed, is sufficient to support normal embryonic development and early larval growth [28].

TABLE I

STEROL CONTENT OF HOUSE-FLY EGGS

	Equivalent per egg (μg)
Total C^{14}-sterols	0.051
Free sterols	0.029
Sterol esters	0.021
Polar compounds	0.001
Cholesterol	0.043
7-Dehydro-cholesterol	0.007

We have also examined the fatty acid moiety of the sterol esters of the eggs and found these to be composed of greater than 92% mono-unsaturated C-16 and C-18 fatty acids, with C-18:1 accounting for about 76% and C-16:1 another 16% [30]. Cholesteryl oleate has recently been reported to be the major sterol ester in the cockroach, Eurycotis floridana [31].

An examination of the sterol content of newly-hatched house-fly larvae has shown that there is little, if any, detectable quantitative change in the sterol content of the eggs and newly-hatched larvae. However, about 80%

of the sterol esters present in the egg are converted to free sterols during embryonic development. Both the cholesterol and 7-dehydro-cholesterol fractions appeared to remain constant [28]. These studies are being continued using high-specific-activity C^{14}-sterols which permit a more precise measurement of both the changes and overall balance of these sterols.

"SPARING" STUDIES

The house fly, like Dermestes [14], may be reared on a larval diet containing sub-minimal quantities of cholesterol plus a "sparing sterol" [32, 33]. We have found that a number of the sterols which "spare" cholesterol in Dermestes, including cholestanol, will perform this same function in house-fly larvae. When added to an aseptic semi-defined larval diet, these sterols may replace greater than 95% of the dietary cholesterol requirement and still provide for optimal growth and development. However, from a number of studies in which we have used different "sparing sterols" and tested various techniques for making the "essential" cholesterol available to the larvae, we have found that the actual "essential" cholesterol requirement may be more of the order of 0.5% of the total sterol requirement, or less than 0.1 μg per insect.

An interesting phenomenon observed in these sparing studies is the efficient selective uptake and/or accumulation of cholesterol from the larval diet by the house fly, even when the cholesterol is present at only a fraction of the concentration of the sparing sterol (Table II). An extreme example of this phenomenon was observed in a study in which we used cholesterol in a 1:10 000 ratio to the "sparing sterol" and found the pupae to contain 80% of the cholesterol available in the larval diet [28].

TABLE II

SELECTIVE UPTAKE AND/OR ACCUMULATION OF C^{14}-CHOLESTEROL FROM THE LARVAL DIET BY HOUSE-FLY LARVAE

Sterol concentration (Dry weight)	C^{14} - sterols per insect		
	Available in diet (μg)	Present in insect (μg)	Total in insects (%)
0.2% Cholestanol + 0.01% C^{14}-cholesterol	3.00	1.72	57.3
+ 0.0005% C^{14}-cholesterol	0.150	0.106	70.7

We have studied the fate of a sparing sterol in house-fly larvae using C^{14}-cholestanol plus sub-minimal quantities of cholesterol in an aseptic semi-defined larval diet. Pupae reared in this manner were found to contain about 14.5 μg of C^{14}-sterol per insect, nearly all of which was present as free sterol. Analysis by reverse-isotope dilution and/or gas-liquid chromatography showed that there was no conversion of cholestanol to cholesterol

and that greater than 99% of the C^{14}-sterol present in the insects represented unchanged cholestanol [33]. The house-fly then differs from two species of cockroaches, Blattella germanica and Eurycotis floridana, which convert nearly half of the cholestanol to Δ^7 -cholestenol during its utilization as a "sparing sterol" [34, 35] and which contain a high percentage of both the cholestanol and Δ^7 -cholestenol in the tissues as sterol esters [15, 36]. It would appear that this conversion of a stanol to a Δ^7 -stenol is related to the dehydrogenation of Δ^5 -sterols such as cholesterol to form their corresponding 5, 7-dienes. We have found this metabolic pathway in both the adult house-fly [29, 37] and the nymphal German cockroach [38], but it occurs at an extremely low level in house-fly larvae [28].

We are currently using C^{14}-cholesterol to study the fate of the minute "essential" cholesterol requirement of house-fly larvae. Our preliminary findings confirm those of CLAYTON et al. [15] in that greater than 95% of the C^{14}-compounds present in pupae and adults reared under sparing conditions, using C^{14}-cholesterol, represent unchanged cholesterol. However, both balance studies on the different developmental stages and the presence of C^{14}-labelled polar metabolites in the larval medium and in the insects suggest that at least a portion of the "essential" cholesterol requirement of house-fly larvae is metabolized during its use for growth and metamorphosis [28], and it is in this area that we are currently concentrating our effort.

UTILIZATION AND METABOLISM OF PHYTOSTEROLS

The literature suggests that a number of the phytophagous and omnivorous insects which utilize the C-28 and C-29 phytosterols convert these sterols to cholestane derivatives. Much of the evidence for such conversions involves either the use of natural diets [39, 40] or identifications which rely on bioassay and/or paper chromatography [41, 42, 43]. However, the use of radiotracer-labelled plant sterols in semi-defined diets and the availability of gas-liquid chromatographic systems which separate with ease the various phytosterols and cholesterol now permit a more critical investigation of this problem. Recently CLARK et al. [44], using C^{14}-labelled ergosterol, have definitely established that the German cockroach (Blattella germanica) can convert this C-28 trienol to 22-dehydro-cholesterol by saturation of the Δ^7 double bond and demethylation of the side chain. In a subsequent study using H^3 -labelled β-sitosterol and gas-liquid chromatographic analysis, we have found the German cockroach to have also available the biochemical mechanism for dealkylating the β-sitosterol side chain to form cholesterol [45].

Since the house-fly is routinely reared on the standard CSMA larval medium, which consists solely of plant material, this insect then must be able to use certain plant sterols to fulfil at least a part of its dietary sterol requirement. Adult flies reared on this medium have been reported to contain a mixture of sterols, and the major sterol(s) present was found to differ from both cholesterol and β-sitosterol in its chemical and physical properties [46, 47]. In a recent study THOMPSON et al. [48] have shown the sterols present in CSMA-reared flies to consist primarily of a mixture of campesterol (74%) and β-sitosterol (21%) and that the campesterol origi-

nates from the CSMA medium through its selective uptake and/or retention by house-fly larvae.

The presence in these insects of a mixture of phytosterols is in contrast to the reports that larvae of Musca domestica vicini convert β-sitosterol to cholesterol [42, 43]. To resolve these differences, we have used H^3-β-sitosterol to study its metabolism in house-fly larvae and adults [37, 49]. When fed in a semi-defined adult diet, the H^3-β-sitosterol was efficiently utilized for egg production. Although the major portion of the radioactive compounds in both the adult flies and eggs behaved as free sterols, as much as 30% of the H^3-sterol in the eggs was esterified. Column chromatographic analysis of the total sterols indicated the presence of both Δ^5 and $\Delta^{5,7}$ sterols. The Δ^5 sterols, which accounted for 88 to 95% of the total H^3-labelled compounds, was found to consist of greater than 90% of β-sitosterol when analysed by gas-liquid chromatography and reverse-isotope dilution. The 5, 7-diene fraction was tentatively identified as 7-dehydro-β-sitosterol using the same techniques. Similar results were obtained with pupae reared aseptically on a semi-defined larval diet containing H^3-β-sitosterol in which greater than 99% of the sterols present behaved like β-sitosterol. There was no detectable conversion of β-sitosterol to cholesterol in either the larvae, the adult fly or the eggs. This was determined by reverse-isotope dilution and purification through the dibromide and by gas-liquid chromatographic analysis with concurrent trapping of the radioactive compounds. The house-fly then uses β-sitosterol directly and as a precursor for 7-dehydro-β-sitosterol, to fulfil at least a part of its sterol requirement, without detectable conversion of this phytosterol to cholesterol.

In the course of these investigations we have observed an interesting physiological difference between adult flies reared on larval diets containing phytosterols and those reared on larval diets containing cholesterol. The adult female house-fly has generally been found to require a complete diet for ovarian maturation and to show little or no ovarian growth on a diet consisting solely of carbohydrates. When flies from our NAIDM strain were reared on the CSMA larval diet, which contains a mixture of phytosterols [48], and were held on an adult diet of sucrose and water, only 1 to 2% of these flies developed mature ovaries. However, when the flies were reared on this larval medium supplemented with cholesterol and held on sucrose, then about half the flies developed mature ovaries [19]. This ovarian maturation occurred in the absence of any mortality, an indication that it was not brought about by the females feeding on dead flies, and eggs from these flies were viable and produced larvae which developed to adults. These results have been confirmed with four other laboratory strains of house flies.

In order to eliminate the possibility that the activity was brought about by micro-organisms, these tests were repeated using a semi-defined larval diet and aseptic rearing techniques. The phytosterol β-sitosterol, which is the major sterol present in the CSMA medium [48], was used for comparison. About 50% of the flies reared on the larval diet containing various concentrations of cholesterol and held on an adult diet of sucrose and water were found to contain mature ovaries, as compared with only about 5% of flies reared on the diet containing β-sitosterol. We are currently attempting to determine whether cholesterol, as such, is responsible for this effect or

whether it serves as a precursor for a sterol metabolite which regulates gonad development.

This involvement of cholesterol in ovarian maturation and yolk deposition points to an interesting role for this sterol in the house-fly, since these physiological processes are known to be under endocrine control in a number of insects [50, 51]. Both this effect, and the minute amount of "essential" cholesterol that is required for either embryonic development or complete metamorphosis, in which there is a 400-500 fold increase in weight, strongly support the current concept that at least a portion of the sterol required by insects serves in a metabolic capacity, perhaps as a hormone or a hormone precursor [14, 22].

(Since the preparation of this paper, the moulting hormone (ecdysone) of insects has been reported to be a sterol (KARLSON, P., HOFFMEISTER, H., HOPPE, W. and HUBER, R., Ann. Chem. Liebigs 662 (1963) 1).

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DISCUSSION

K. HAGEN: Do you have any information on, or has your work shown any correlation or relationship between, choline and sterol requirements? It appears that we can get good egg production and fertility without cholesterol or sterol in the adult diet.

W.E. ROBBINS: No, we do not have such information. When it emerges, the female fly probably has from 10 to 15 μg of sterol. If we hold flies for a prolonged period and collect the eggs, we notice that the sterol content goes down. Probably at least half the sterol can be used for egg production. It might take some time to detect any cholesterol deficiency because the storage mechanism is very good, but the house-fly puts a lot of sterol into the first egg and if she runs out, you very soon get non-viable eggs. However, 13 000 μg for egg hatch and larval viability is not the minimum. You could probably go far below that. When Drosophila was studied, it was at first thought that it did not require sterol at all, but when the casein was very carefully extracted it was found that it did. In some of these Diptera, however, there may actually be enough in the protein source. It is a very active material, you know, and only a tenth of a microgram is required to carry an insect to a five- or six-hundredfold increase in mass. When we

extract it from the casein we do not refer to a sterol-free casein, but to a sterol-deficient casein, because though it contains less than one microgram per gram, it is even then not sterol-free. The sterol is firmly bound in it. The same is true of soya-bean protein. I do not know what you were using as a source of protein, but you may have been giving a little sterol along with it.

W. KLOFT: You mentioned that certain of the more primitive insects may be capable of some degree of cholesterol biosynthesis. Dr. G. H. Schmidt of Würzburg has worked out a qualitative and quantitative chemical analysis (proteins, carbohydrates and total lipoids) for the ant Formica polyctena Foerst. During the metamorphosis, which is divided into 10 stages, beginning with prenymphs and ending with young imagoes, he analysed females, workers and males. He found an increase in sterol content during metamorphosis only in workers, although they did not take up any external food. He had to conclude that in this differentiated social hymenopterum workers may have regained the possibility of biosynthesizing sterols, which appears to have been lost in the course of evolution to higher insects. We have no knowledge of the existence of symbiotic bacteria. They seem to have been lost, but I myself am not yet certain whether this is true. Your special tracer techniques may be able to throw light on this problem.

W.E. ROBBINS: That is a very interesting observation. However, as you are probably aware, the subject of cholesterol biosynthesis in primitive insects needs considerably more study. The use of radioactive precursors, I agree, should be very helpful in resolving your problem, but care should be taken to avoid contamination due to the biosynthesis of sterols by symbionts or intestinal micro-organisms. Axenic culture methods, antibiotics or the injection of labelled precursors should be employed.

In another series of experiments on the biosynthesis of cholesterol, we took another species of Thysanura and by-passed the intestinal tract by injection. In our preliminary studies we found no incorporation of injected C^{14}-acetate into the sterols. We did, however, obtain good incorporation into the total lipids.

J. HALBERSTADT: Was gas-liquid chromatography (GLC) used as a separation or isolation tool for the reverse isotope dilution analysis?

W.E. ROBBINS: Most of the gas-liquid chromatographic columns for sterols are low capacity, in the microgram to fraction-of-a-milligram range, because of the low percentages of liquid phase used. Because of this, adsorption chromatography is usually used for separation and isolation. However we do analyse our radioactive samples by GLC with concurrent trapping of the radioactive compounds.

J. HALBERSTADT: Is there any indication as to what the mechanisms might be that cause the efficient selection and/or accumulation of cholesterol as observed in sparing studies?

W.E. ROBBINS: We have not investigated this phenomenon as yet but plan to do so in the very near future.

TYROSINE METABOLISM IN THE BLOWFLY, CALLIPHORA ERYTHROCEPHALA

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Abstract — Résumé — Аннотация — Resumen

TYROSINE METABOLISM IN THE BLOWFLY, CALLIPHORA ERYTHROCEPHALA. Sclerotization, i. e. the transformation of the soft, white larval cuticle into the hardened, dark puparium, is due to interaction of o-quinones with the cuticle proteins. Using radioactively labelled amino-acids, it has been shown that N-acetyldopamine is the immediate precursor of the sclerotizing quinones in Calliphora erythrocephala. The intermediate steps in the biosynthesis of N-acetyldopamine are hydroxylation of tyrosine to dopa, decarboxylation of dopa to dopamine and N-acetylation of dopamine to N-acetyldopamine. This metabolic pathway of tyrosine is followed only in the final-instar larvae; early third-instar larvae catabolize tyrosine by transamination to p-hydroxyphenylpyruvic acid and reduction to p-hydroxyphenyllactic and -propionic acid. The metabolic shift from transamination to hydroxylation and decarboxylation is brought about by the hormone of the prothoracic gland, ecdysone, and can be inhibited by ligation or destruction of the ring gland.

Injection of ecdysone into the ligated animals leads within 10-14 h to activation of the dopadecarboxylase, presumably as the result of biosynthesis of enzyme protein. The action of the hormone is not a direct one on the biosynthetic mechanism, but indirect, the first action of the hormone being on the chromosomes (puffing phenomenon). The working hypothesis is that the hormone interacting with the genetic material leads to stimulation of the synthesis of specific (messenger-) RNA which is transferred to the cytoplasm and produces enzyme proteins.

MÉTABOLISME DE LA TYROSINE DANS LA CALLIPHORA ERYTHROCEPHALA. L'induration, c'est-à-dire la transformation de la cuticule larvaire, douce et blanche, en un puparium dur et sombre, est due à l'interaction d'ortho-quinones avec les protéines de la cuticule. Grâce à l'emploi d'acides aminés marqués au moyen de substances radioactives, on a montré que la N-acétyl-dopamine est le précurseur immédiat des quinones produisant l'induration dans la Calliphora erythrocephala. Les stades intermédiaires de la biosynthèse de la N-acétyl-dopamine sont l'hydroxylation de la tyrosine, qui se transforme en dioxyphénylalamine (dopa), la décarboxylation de la dopa en dopamine et la N-acétylation de la dopamine en N-acétyl-dopamine. Ce processus métabolique de la tyrosine ne se produit qu'au dernier stade de l'état larvaire; les trois premiers stades produisent le catabolisme de la tyrosine par transamination en acide p-hydroxyphényl-pyruvique et réduction en acide p-hydroxyphényl-lactique et propionique. Le processus métabolique de la transamination à l'hydroxylation est provoqué par l'hormone de la glande prothoracique, l'ecdyson, et peut être rendu impossible par la ligature ou l'ablation de la glande annulaire.

L'injection d'ecdyson aux animaux ligaturés entraîne, au bout de 10-14 h, l'activation de la décarboxylase de la dopa, due vraisemblablement à la bioxynthèse de la protéine de l'enzyme. L'action de l'hormone sur le mécanisme de biosynthèse est indirecte, le premier effet de l'hormone étant d'agir sur les chromosomes (phénomène de gonflage). L'hypothèse de travail est que l'hormone qui entre en interaction avec la matière génétique a pour effet de stimuler la synthèse de l'ARN spécifique (vecteur) qui est transmis au cytoplasme et produit les protéines d'enzymes.

МЕТАБОЛИЗМ ТИРОЗИНА У МЯСНОЙ МУХИ, CALLIPHORA ERYTHROCEPHALA. Склеротизация, т.е. превращение мягкой белой кутикулы личинки в затвердевший темный пупарий, происходит благодаря взаимодействию ортохинонов с белками кутикулы. С помощью меченных изотопами аминокислот было показано, что N-ацетилдопамин является ближайшим предшественником склеротизирующих хинонов у Calliphora erythrocephala. Промежуточными стадиями в биосинтезе N-ацетилдопамина являются гидроксилирование тирозина в допа, декарбоксилирование допа до допамина и N-ацетилирование допамина до N-ацетилдопамина. Этот метаболический процесс превращения тирозина происходит только у личинок на последней стадии развития. У личинок в ранней фазе третьей стадии происходит катаболизация ти-

розина путем переаминирования в пара-оксифенилпировиноградную кислоту и восстановление в пара-оксифенилмолочную пара-оксифенилпропионовую кислоту. Метаболическое смещение от переаминирования до гидроксилизации и декарбоксилизации вызывается гормоном переднегрудной железы, экдисоном, и может задерживаться наложением лигатуры и разрушением кольцевой железы.

Введение экдисона животным с наложенной лигатурой приводит в течение 10 - 14 часов к активации допадекарбоксилазы, вероятно в результате биосинтеза ферментативного белка. Гормон оказывает не прямое, а косвенное действие на механизм биосинтеза и, прежде всего, на хромосомы (явление отечности). Рабочая гипотеза заключается в том, что гормон, взаимодействуя с генетическим материалом, приводит к стимулированию синтеза особой (переносной) РНК, которая переносится в клеточную протоплазму и производит ферментативные белки.

METABOLISMO DE LA TIROSINA EN LA MOSCA CALLIPHORA ERYTHROCEPHALA. La esclerotización (transformación de la cutícula blanda y blanca de la larva en la cubierta dura y oscura de la ninfa) se debe a la acción recíproca de o-quinonas con las proteínas de la cutícula. Empleando aminoácidos marcados se ha comprobado que la N-acetildopamina es el precursor inmediato de las quinonas esclerotizantes en la Calliphora erythrocephala. Las etapas intermedias en la biosíntesis de la N-acetildopamina son la hidroxilación de la tirosina que da dioxifenil-alanina, la descarboxilación de ésta que da dopamina, y la N-acetilación de la dopamina que da la N-acetildopamina. Este proceso metabólico de la tirosina se produce solamente en las larvas del último estadio; al principio del tercer estadio las larvas catabolizan tirosina por transaminación, dando ácido p-hidroxifenilpirúvico, y por reducción, dando los ácidos p-hidroxifenillácticо y p-hidroxifenil propiónico. Esta transición metabólica desde la transaminación a la hidroxilación y descarboxilación se debe a la hormona de la glándula protorácica (ecdyson) y se puede inhibir ligando o destruyendo la glándula anular.

Si se inyecta ecdyson en los animales ligados se produce al cabo de 10 a 14 h una activación de la dopadecarboxilasa, debida probablemente a la biosíntesis de proteínas enzimáticas. La acción de la hormona sobre el mecanismo de biosíntesis no es directa sino indirecta, dejándose sentir sus primeros efectos sobre los cromosomas (fenómeno de turgencia). La hipótesis de que se partió es que la hormona que actúa sobre las sustancias genéticas y que sufre la acción de éstas estimula la síntesis de un ARN específico («vehículo») que se traslada al citoplasma y produce proteínas enzimáticas.

1. TYROSINE AND SCLEROTIZATION

Interest in tyrosine metabolism in insects arose mainly in connection with sclerotization, i.e. the hardening and darkening of the larval cuticle during pupation [1]. This process is brought about by the interaction of o-quinones with the cuticular proteins [2]. These quinones arise from the respective o-diphenols which are oxidized in the cuticle by the polyphenol-oxidase present there. Many such phenolic substances, particularly acids, were extracted from cuticles of different insects and their structural relationship to tyrosine pointed to this amino-acid as the precursor of the phenols. A more direct indication of the involvement of tyrosine in the sclerotization process was its high rate of incorporation in the cuticle of pupating larvae, whereas other amino-acids, for example leucine, were hardly incorporated at all [3].

2. TYROSINE METABOLISM IN LATE III-INSTAR LARVAE

We injected generally labelled C^{14}-tyrosine into final-instar Calliphora larvae and looked for radioactive metabolites that might be potential sclerotizing agents on paper chromatograms. The main metabolite was identified as N-acetyldopamine [4]. Its role in sclerotization was established

by the high incorporation of injected 2'C^{14}-labelled N-acetyldopamine in the cuticle of pupating larvae and also by following the in vivo concentration of this metabolite during larval development [5]. The concentration is low in early III-instar larvae, rises in the late III-instar larvae and disappears during pupation.

Using larval homogenates as the enzyme source, we studied the biosynthesis of N-acetyldopamine [6]. The first reaction is the hydroxylation of tyrosine to DOPA. This hydroxylation is brought about by a particle-bound enzyme system whose action is potentiated by the presence of H-donors, such as DPNH or ascorbic acid. The next step is the decarboxylation of DOPA to dopamine by a pyridoxal-phosphate-dependent decarboxylase in the epidermis cells. Dopamine is further acetylated by a transacetylase system and thus protected from ring closure, which is so common for this class of compounds [7]. Most of the N-acetyldopamine thus formed will be used for puparium formation, while a small part is being stored up as an o-glucoside which plays a role later on in the eclosion of the imago.

3. TYROSINE METABOLISM IN EARLY III-INSTAR LARVAE

The injection of tyrosine into early III-instar larvae showed a different "spectrum" of metabolites: phenol carbonic acids (such as p-hydroxyphenylpropionic acid) were isolated [6]. The incubation of tyrosine with Calliphora homogenates also gave p-hydroxyphenylpropionic acid as a main product. From the incubation mixtures p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid were isolated so that the pathway is tyrosine → p-hydroxyphenylpyruvic acid → p-hydroxyphenyllactic acid → p-hydroxyphenylpropionic acid. The first reaction was studied in greater detail and was shown to be a transamination reaction with α-ketoglutarate as amino-acceptor and pyridoxal phosphate as coenzyme. In Fig. 1 we have summarized the metabolism of tyrosine in early and late III-instar Calliphora larvae.

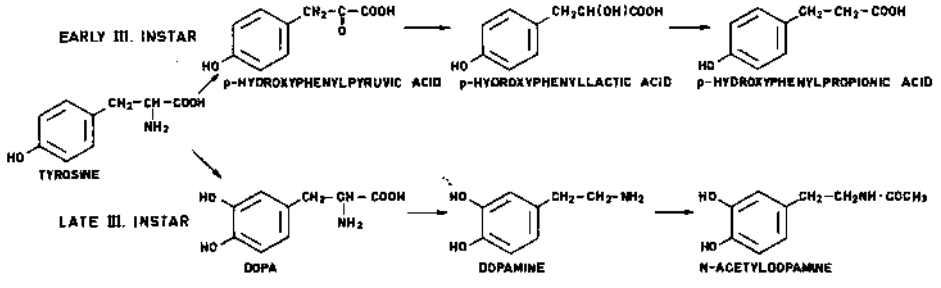


Fig. 1

Tyrosine metabolism in early and late III-instar Calliphora larvae (from KARLSON and SEKERIS [5]).

4. CONTROL OF TYROSINE METABOLISM BY ECDYSONE

The metabolic shift from transamination to hydroxylation and decarboxylation coincides with the time of secretion of the hormone ecdysone. Ecdy-

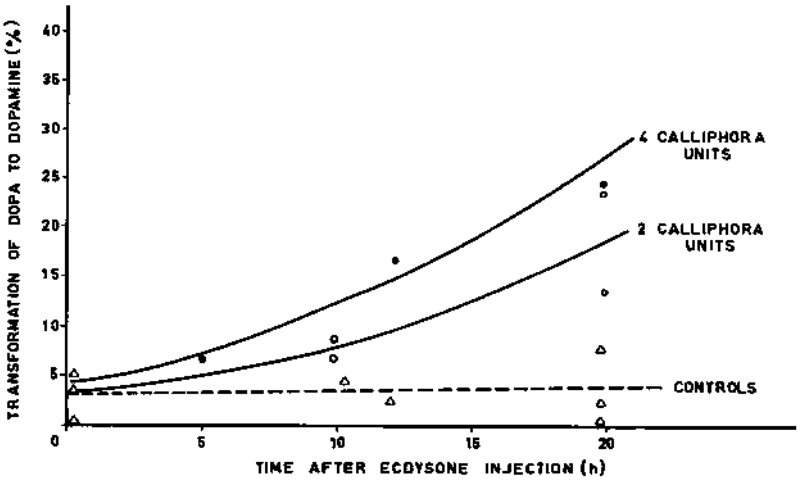


Fig. 2

DOPA decarboxylase activity in relation to time after injection of ecdysone
– –Δ– –Δ– – ligated control larvae
—o—o— injected with 2 Calliphora Units
—•—•— injected with 4 Calliphora Units
(from KARLSON and SEKERIS [9]).

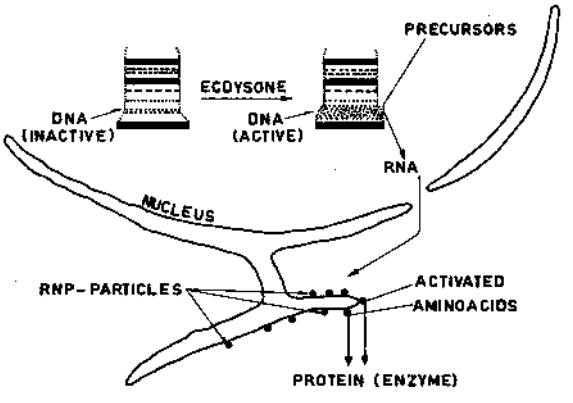
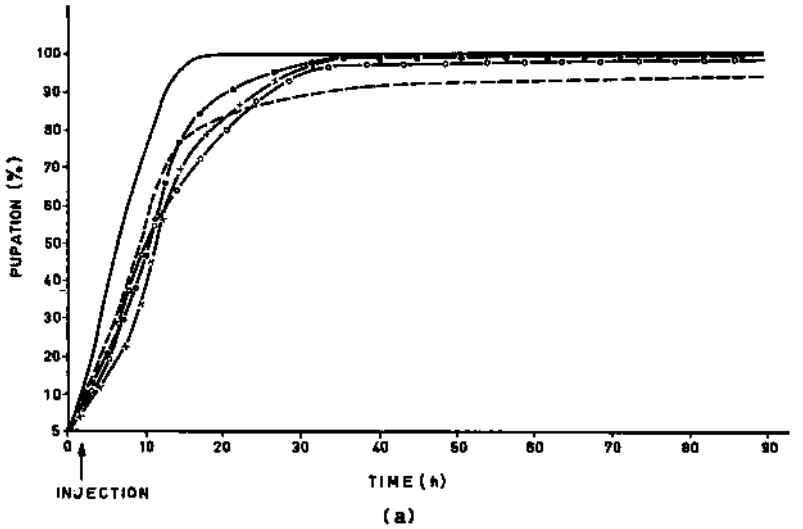


Fig. 3

Schematic representation of the mode of action of hormones
The primary action of the hormone is the activation of gene loci (DNA) which leads to formation of nuclear (messenger) RNA from nucleic acid precursors. The RNA formed enters the cytoplasm and attaches itself to the ribosomal surface, thus serving as a template for specific protein (enzyme) synthesis.
(from KARLSON [12]).



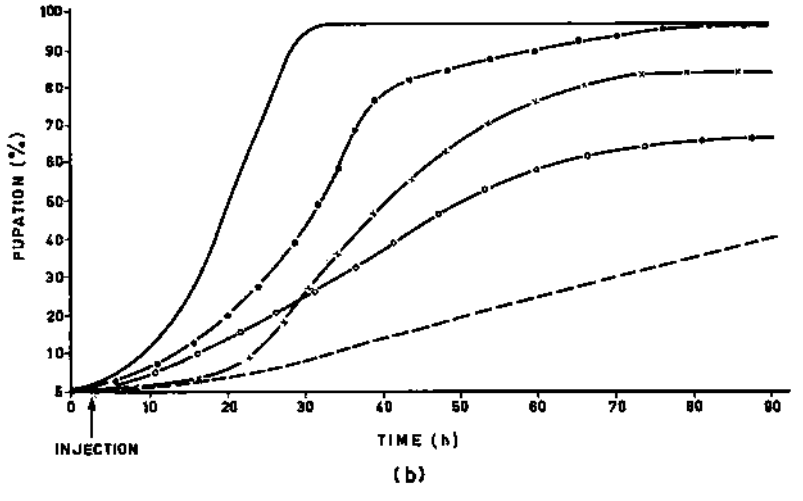


Fig. 4

Influence of antimetabolites on the pupation rate of Calliphora larvae
Groups of 40 larvae were injected with 4 different antimetabolites (concentration in brackets) and their pupation rate was followed in comparison with that of untreated larvae.

——— untreated larvae
—o—o— larvae injected with streptomycin (300 µg)
—x—x— larvae injected with puromycin (6 µg)
- - - - - - larvae injected with actinomycin (0,02 µg)
—•—•— larvae injected with mitomycin (0.1 µg)

In Fig. 4(a) the substances were injected during the last day of larval life, in Fig. 4(b) 1-2 days before pupation.

sone, the product of the prothoracic or ring gland, induces a series of changes which lead to pupation [8]. It is natural to conclude that the shift in the metabolism of tyrosine is related in some way with the secretion of the hormone. Ligation of late III-instar larvae prevents pupation; the cuticle remains white and soft, thus retaining its larval character. The concentration of the sclerotizing agent N-acetyldopamine in the ligated animals remains low in comparison with the normal larvae, which points to an impaired synthesis. We studied the activity of the enzymes involved in the biosynthesis of N-acetyldopamine in these animals and found that the activity of the hydroxylase and decarboxylase remained low [9]. The injection of ecdysone leads to activation of the enzymes, as seen in Fig. 2 with reference to decarboxylase. This activation is dose-dependent and cannot be clearly seen until 8-10 h after injection of the hormone, i.e. the effect is a late one.

5. NATURE OF ECDYSONE ACTION

This induction of enzyme activity by ecdysone brings up the question of the nature of the activation: does the hormone activate a pre-existing inactive proenzyme, is it a de novo synthesis of enzyme protein, an action on a coenzyme, etc.? A new approach to this problem has been opened up by the work of CLEVER and KARLSON [10]. Injection of ecdysone into final-instar Chironomus larvae leads within 30 min to modification of the structure of the salivary gland chromosomes. These morphologically modified gene loci, known as "puffs", are active centres of RNA formation. This was shown by autoradiography of salivary gland cells of Chironomus after injecting H^3-uridine or H^3-cytidine [11]. Recalling the role of nuclear RNA in protein synthesis, we can picture the action of the hormone indirectly on protein synthesis through activation of DNA → messenger RNA synthesis → protein (enzyme) synthesis (Fig. 3) [12].

We have indirect evidence that de novo synthesis of enzyme protein takes place. Injection into Calliphora of substances which inhibit protein formation (such as puromycin or streptomycin) leads to delayed pupation and also to inhibition of decarboxylase induction. No significant inhibition can be seen if the substances are given in the last 10-15 h of larval life, while maximum effectiveness is observed if they are injected 24-48 h before pupation, at the time of hormone secretion when the activation of the biosynthetic chain begins (see Fig. 4). Similar action is exhibited by some DNA and RNA inhibitors (such as actinomycin, mitomycin and 5-F-deoxyuridine), which fits in with the hormone action mechanism proposed.

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DISCUSSION

R. von BORSTEL: The conclusions from the work of Clever and Karlson must be approached with caution, since recent work by H. Kroeger in Zürich indicates that the same "puff" that can be activated by ecdysone can also be activated by several different narcotics as well as butanol. Even though the ecdysone activation of the puff occurs at extremely low concentrations of the compound, the effect may still be a secondary one.

C. E. SEKERIS: Ecdysone induces the puffing phenomenon in concentrations of about 0.01 μg, that is with minute amounts of substance, which points to the specific nature of the effect. There is a possibility that ecdysone does not act directly on the puff but indirectly by combining with a repressor (according to the theory of Jacob et al). This still remains to be proved.

S. FUZEAU-BRAESCH: Do you think that N-acetyldopamine is responsible for hardening and pigmentation or just for one of these? Can you give reasons?

C. E. SEKERIS: In Calliphora sclerotization and pigmentation cannot be separated. We believe that N-acetyldopamine is the sclerotizing agent in Calliphora and that, during the quinone tanning, it also gives the dark colour to the puparium. Of course this is not true of all insects and your paper has a bearing on this problem.

ÉTUDE DE LA PIGMENTATION TÉGUMENTAIRE DES INSECTES A L'AIDE DE RADIOÉLÉMENTS

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Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPE STUDY OF TEGUMENTARY PIGMENTATION IN INSECTS. The nature of insect cuticle, which is made up in large part of scleroproteins, calls for the use of labelled isotopes to obtain answers to certain questions regarding pigmentation.

The following method, which has the advantage of being quick and easy to apply, has been developed.

The labelled substance chosen is injected into the animal at various phases of its skin-shedding cycle: before secretion of the cuticular proteins, i. e. when the cuticle is at rest; at the time these proteins are deposited; and, lastly, at the time their sclerification begins.

After a suitable interval the cuticle is removed, suitably treated, and subjected to full autoradiography. Photographic comparison of the results then indicates whether or not the substance chosen has been used for any formation of pigment, due account being taken of prior chemical processes involving the substance.

The findings presented in the paper relate to three labelled substances: two carbon-14 amino-acids - tyrosine and tryptophane - and inorganic sulphur-35 in the form of sodium sulphate. It has thus been possible to give direct proof of the origin of variously-coloured cuticular pigments and to discuss the role of tryptophane and sulphur in forming pigments in insect integument.

All cuticular pigments spring from the metabolism of tyrosine, thus confirming the term "melanic" hitherto applied to them without direct proof. Tryptophane, exceptionally integrated in the cuticle, is the substratum of the ommochromic red and black pigments in the hypodermis. Inorganic sulphur plays no regular specific role in the formation of cuticular pigments, contrary to what has been suggested by various hypotheses on the role of the sulphydryl group.

From the standpoint of comparative biochemistry, the melanins appear to be purely cuticular among insects, granular melanins being confined to the vertebrates. Dark hypodermic granules in insects are ommochromic, derived from tryptophane, and not melanic.

ÉTUDE DE LA PIGMENTATION TÉGUMENTAIRE DES INSECTES A L'AIDE DE RADIOÉLÉMENTS. En raison de la nature de la cuticule des insectes - constituée en grande partie de scléroprotéines - il est nécessaire, pour répondre à certaines questions relatives à la pigmentation, d'utiliser des éléments marqués.

La méthode suivante a été mise au point; elle présente l'avantage de la rapidité et de la facilité.

L'élément choisi est injecté à l'animal à différents moments de son cycle de mue: avant la sécrétion des protéines cuticulaires, la cuticule étant alors au repos; au moment du dépôt de ces protéines; à l'instant où commence le processus de sclérification de ces protéines.

Après un laps de temps adéquate la cuticule est prélevée, traitée de façon appropriée et autoradiographiée in toto. La comparaison photographique des résultats permet de conclure à l'utilisation ou non de l'élément choisi dans telle formation pigmentaire, compte tenu des processus chimiques antérieurs relatifs à l'élément.

Les résultats présentés concernent trois éléments marqués: deux acides aminés - tyrosine et tryptophane - au carbone-14, du soufre-35 inorganique sous forme de sulfate de sodium. On a pu ainsi démontrer directement l'origine des pigments cuticulaires de différentes couleurs et discuter le rôle du tryptophane et du soufre dans les formations pigmentaires du tégument des insectes.

Les pigments cuticulaires sont tous issus du métabolisme de la tyrosine, confirmant le terme de «mélaniques» qui leur était jusqu'à présent attribué sans preuve directe. Le tryptophane, exceptionnellement intégré dans la cuticule, constitue le substrat des pigments noirs et rouges ommochromiques de l'hypoderme. Le soufre inorganique ne joue pas de rôle spécifique régulier dans la formation des pigments cuticulaires, contrairement à ce que laissaient supposer différentes hypothèses sur le rôle des groupements sulfhydriles.

Du point de vue de la biochimie comparée, les mélanines semblent être chez les insectes exclusivement cuticulaires, les granulaires étant réservées aux vertébrés. Quant aux granules hypodermiques sombres, ils sont, chez les insectes, de nature ommochromique, dérivés du tryptophane et non mélaniques.

ИЗУЧЕНИЕ ПИГМЕНТАЦИИ ОБОЛОЧКИ НАСЕКОМЫХ С ПОМОЩЬЮ РАДИОЭЛЕМЕНТОВ. В связи с характером кутикулы насекомых, состоящей в значительной части из склеропротеина, возникает необходимость в использовании меченых элементов для изучения некоторых процессов пигментации.

Был использован следующий быстрый и легкий метод исследования. Определенный элемент вводится животному в различные периоды линьки: до выделения кутикулярных протеинов, когда кутикула находится в стадии покоя, в момент отложения этих протеинов и, наконец, в момент, когда начинается процесс склеротизации этих протеинов.

Через определенный промежуток времени кутикула снимается, обрабатывается соответствующим образом и в целом подвергается авторадиографии. Фотографическое сравнение полученных результатов позволяет прийти к выводу относительно использования или неиспользования данного элемента в образовании пигмента с учетом предыдущих химических процессов, касающихся элемента.

Представленные здесь результаты относятся к трем меченым элементам: двум аминокислотам - тирозину и триптофану, меченным углеродом-14, и неорганической сере-35 в виде сульфата натрия. Таким образом представилось возможным наглядно показать происхождение кожных пигментов различных цветов и обсудить роль триптофана и серы в образовании пигмента оболочки насекомых.

Кутикулярные пигменты образуются в результате метаболизма тирозина, подтверждая тем самым термин "меланиновые", который был им до сих пор присвоен без прямого доказательства. Триптофан, который входит только в состав кутикулы, представляет собой оммохромный субстрат черных и красных пигментов гиподермы. Неорганическая сера не играет определенной специфической роли в образовании кутикулярных пигментов вопреки различным гипотезам о роли сероводородных соединений.

С точки зрения сравнительной биохимии меланины присутствуют, по-видимому, только у кутикулярных насекомых, а зернистость свойственна позвоночным. Что касается темных гиподермических гранул, то у насекомых они носят оммохромный характер, лишены триптофана и не относятся к меланинам.

ESTUDIO DE LA PIGMENTACIÓN TEGUMENTARIA DE LOS INSECTOS CON AYUDA DE RADIOELEMENTOS. La naturaleza de la cutícula de los insectos -constituida en gran parte por escleroproteínas- obliga a utilizar elementos marcados para resolver ciertas cuestiones relativas a la pigmentación.

Se ha preparado el método siguiente, que ofrece la ventaja de su rapidez y simplicidad.

El elemento elegido se inyecta en el animal en diferentes momentos de su ciclo de muda: antes de la secreción de las proteínas cuticulares, cuando la cutícula se encuentra en reposo; en el momento de depositarse estas proteínas, y, por último, en el instante en que comienza el proceso de esclerificación de dichas proteínas.

Después de un lapso de tiempo adecuado se retira la cutícula, se somete a un tratamiento apropiado y se autorradiografía in toto. La comparación fotográfica de los resultados permite determinar si el elemento elegido ha sido utilizado o no en la formación pigmentaria, teniendo en cuenta los procesos químicos anteriores que afectan a dicho elemento.

Los resultados presentados en la memoria se refieren a tres elementos marcados: dos aminoácidos -tirosina y triptófano- marcados con carbono-14, y azufre-35 inorgánico, en forma de sulfato de sodio. Con este procedimiento se ha podido demostrar directamente el origen de los pigmentos cuticulares de diferentes colores y analizar la función del triptófano y del azufre en las formaciones pigmentarias del tegumento de los insectos.

Los pigmentos cuticulares provienen todos del metabolismo de la tirosina, lo que confirma el término de «melánicos» que hasta ahora se les atribuía sin pruebas directas. El triptófano, excepcionalmente integrado en la cutícula, constituye el substrato de los pigmentos negros y rojos homocrómicos de la hipodermis. El azufre inorgánico no desempeña una función específica regular en la formación de los pigmentos cuticulares, contrariamente a lo que permitían suponer diferentes hipótesis sobre la función de los grupos sulfhidrilos.

Desde el punto de vista de la bioquímica comparada, las melaninas parecen ser exclusivamente cuticulares en los insectos, mientras que son granulares en los vertebrados. En cuanto a los gránulos hipodérmicos oscuros, en los insectos son de naturaleza homocrómica, derivados del triptófano, y no melánica.

Un certain nombre d'éléments radioactifs sont utilisés dans les marquages d'insectes. Il est intéressant de connaître le sort réservé à ces

éléments en réalisant la détection non globalement mais, pour la partie externe, par autoradiographie. Etant donné le cycle de mue fondamental de l'insecte et son métabolisme particulier en rapport avec la formation de la nouvelle cuticule, on doit distinguer des étapes et rechercher les variations dans l'incorporation des substances au cours de ce cycle. Une telle étude est réalisée ici pour le soufre à l'occasion d'une analyse du tégument dont les formations pigmentaires étudiées à l'aide de tyrosine et tryptophane radioactifs vont servir de base de comparaison.

MÉTHODE

La méthode utilisée d'autoradiographie in toto présente l'avantage de la rapidité et de la facilité. Si elle ne fournit pas de données quantitatives précises, elle permet d'atteindre par contre un niveau que l'analyse chimique ne peut approcher: celui des différences dans la structure fine, pigmentaire ou morphologique de la cuticule.

L'élément choisi est injecté à l'insecte dans la cavité générale, dissout dans du liquide de Ringer pour insecte, en quantité variant de 10 à 20 μl représentant une activité totale voisine du microcurie. L'animal est endormi au gaz carbonique et l'injection effectuée dans l'abdomen, à travers une membrane intersegmentaire, à l'aide d'une micropipette de verre.

Les injections sont réalisées à des moments variés du cycle de mue:

a) avant la sécrétion des protéines cuticulaires, lorsque la cuticule est au repos,
b) au moment du dépôt de ces protéines avant la mue,
c) à l'instant ou commence le processus de sclérification et de pigmentation de ces protéines.

Après un laps de temps adéquat, la cuticule est prélevée, nettoyée à la loupe binoculaire, traitée rapidement de façon appropriée par une solution de potasse bouillante qui élimine toute trace de tissus sous-jacents (hypoderme), puis lavée, séchée, et autoradiographiée sur film spécial «Radio dentaire KODAK». L'autoradiogramme donne l'image des régions radioactives, que l'on peut comparer avec la cuticule elle-même, ou avec une photographie de celle-ci lorsqu'il s'agit d'étudier de près les dessins pigmentaires. Un exemple des résultats obtenus est présenté en figure 1.

Les substances suivantes ont été utilisées:

1. l - Tyrosine, marquée uniformément au ^{14}C (Amersham, Radiochemical Centre).
2. Tryptophane, marqué au ^{14}C, premier atome de la chaîne (Amersham, Radiochemical Centre).
3. Sulfate de sodium marqué au ^{35}S (C.E.A.)
4. dl - méthionine marqué au ^{35}S (C.E.A.)
5. dl - cystine marqué au ^{35}S (C.E.A.)

Les animaux utilisés sont des insectes hétérométaboles: Gryllus bimaculatus de Geer (gryllidae, orthoptères), (substances 1- 5), et Locusta migratoria (acrididae) (substance 1).

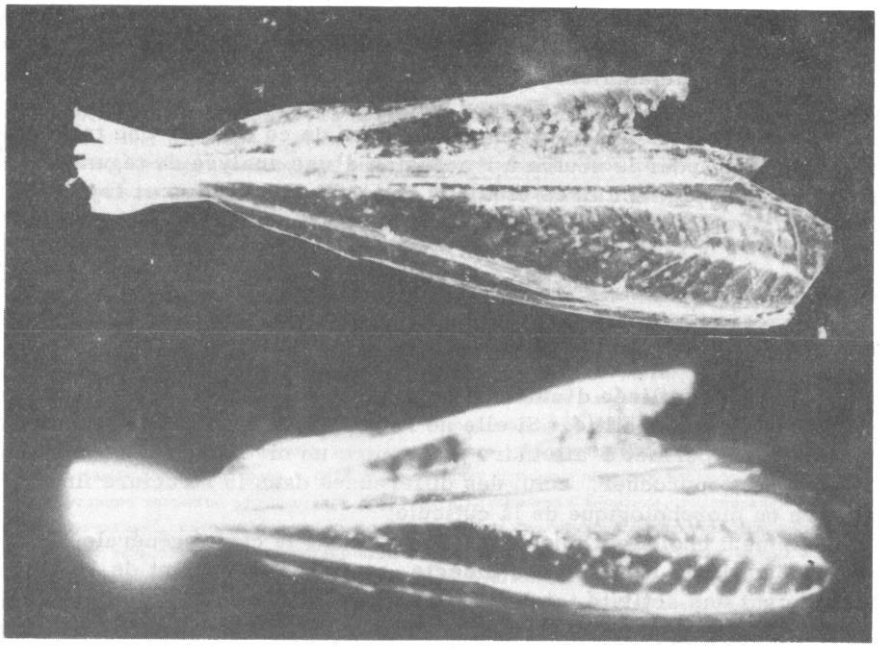

Figure 1

Exemple d'étude chromatographique de la cuticule: incorporation de tyrosine radioactive post-éxuviale dans le fémur postérieur de Locusta migratoria.
A gauche: image des deux faces du fémur; en blanc, les régions pigmentées.
A droite: autoradiogramme correspondant.

FORMATION DE LA CUTICULE ET PIGMENTATION

Il est bon de rappeler ici l'essentiel des processus de formation de la cuticule chez les insectes.

Avant la mue et sous l'ancienne cuticule se constitue une couche de protéines sécrétée par les cellules de l'hypoderme sous-jacent; ce sont elles qui deviendront la partie solide de la future cuticule. Molles et incolores avant la mue, aussitôt après le rejet de l'ancienne cuticule ces protéines sont durcies, sclérifiées selon un processus complexe de «tannage» par des substances quinoniques, en même temps qu'elles deviennent pigmentées.

Une étude de la cuticule générale de l'ensemble du corps a été effectuée; parallèlement à celle-ci une observation plus détaillée a été réalisée dans une région intéressante à cause du décalage chronologique de sa formation: il s'agit de deux processus latéraux du condyle de l'articulation fémur-postérieur/tibia, petites zones en forme de croissant renforçant l'articulation en rapport avec la fonction saltatrice de la patte, qui durcissent et se pigmentent avant la mue. Enfin, une attention spéciale a également été accordée aux grosses épines du tibia postérieur.

Il faut dès le départ distinguer l'incorporation des substances:

1° dans les protéines cuticulaires mises en place avant la mue (injection: un ou deux jours avant la mue, - prélèvement à l'éxuviation ou après la mue, à condition de tenir compte éventuellement de l'activité propre à la période qui suit la mue);

2° après la mue au moment du durcissement et de la pigmentation (injection: à la mue, - prélèvement après durcissement: un jour).

3° dans la cuticule au repos (injection entre les deux mues plus de deux jours après la mue précédente et au moins trois jours avant la mue suivante, prélèvement un ou deux jours après).

Le tableau I schématise les résultats obtenus.

1. Tyrosine et tryptophane dans la cuticule

La tyrosine est incorporée dans les protéines cuticulaires d'une façon uniforme. Par contre, après la mue, elle l'est uniquement dans les zones pigmentées, quelle que soit la couleur du pigment. Les autoradiogrammes montrent une concordance parfaite entre les régions radioactives et les zones pigmentées de la cuticule, tandis que les zones durcies sans pigmentation sont dénuées de radioactivité quelle que soit l'espèce considérée, les animaux ayant vécus groupés ou isolés (fig. 1). La fraction pré-éxuviale de la tyrosine est protéinogène, tandis que la fraction post-éxuviale est pigmentogène.

Les zones non pigmentées de la cuticule durcissent apparemment autant que les zones pigmentées, bien qu'elles n'intègrent pas de tyrosine après la mue. Le processus chimique de ce durcissement n'est pas établi. On voit donc que la fraction post-éxuviale pigmentogène de la tyrosine ne peut être mise en évidence que par la comparaison des autoradiogrammes pré- et post-éxuviaux et doit passer inaperçue à l'analyse chimique globale. C'est peut-être la raison pour laquelle KARLSON et coll. [2] n'ait pas trouvé de différences très nettes entre les insectes mélanisés, Schistocerca gregaria et ceux d'une souche «albino» dépigmentés de la même espèce, tandis qu'ici la concordance est toujours parfaite entre tyrosine et mélanine lorsque l'étendue de cette dernière varie, par exemple sous l'influence du groupement.

Dans le processus du condyle, l'incorporation se fait uniquement et fortement avant la mue, attestant bien de l'achèvement précoce inhabituel de cette zone. Les épines du tibia ne présentent pas de caractères distinctifs.

Le tryptophane est lui aussi intégré dans les protéines cuticulaires; il joue, par contre, fort peu de rôle dans les processus qui se déroulent après la mue et n'est pas en rapport évident avec la pigmentation.

Dans la cuticule au repos, aucun des deux acides aminés n'est incorporé.

Par contre le tryptophane est fortement intégré aux granules d'ommochromes, pigments hypodermiques sous-jacents, dont il est la substance-mère, ce qui permet de distinguer parfaitement ces granules des granules mélaniques issus de la tyrosine tels qu'on les connaît chez les vertébrés, et qui jusqu'à présent n'ont pas été trouvés chez les insectes. Chez ces derniers les pigments polymérisés issus de la tyrosine semblent bien être

TABLEAU I

RADIOACTIVITÉ DE LA CUTICULE APRÈS INJECTIONS DE DIFFÉRENTES SUBSTANCES RADIOACTIVES EN FONCTION DU CYCLE DE MUE

Substance radioactive injectée	Radioactivité pré-exuviale des protéines cuticulaires (Injections avant la mue)			Radioactivité post-exuviale de la cuticule (Injections à la mue)			Radioactivité de la cuticule au repos (Injections entre deux mues)
	Cuticule générale	Processus du condyle	Epines tibiales	Cuticule générale	Processus du condyle	Epines tibiales	
Tyrosine (^{14}C)	+++	++++	++	++++ (Pigments)	0	+	0
Tryptophane (^{14}C)	++	+	+	+ 0	0	+ 0	0
Sulfate de sodium (^{35}S)	+	0	+	++	+	+++	+
Méthionine (^{35}S)	++++	++	+	++	++	+	+
Cystine (^{35}S)	+	++	+	++	++	+	+

Détection effectuée par autoradiographie: nombre de croix (+) = évaluation visuelle approximative des intensités.

l'unique apanage de la cuticule au sein de laquelle ils se trouvent à l'état diffus et non granulaire [1].

2. Analyse comparée du rôle du soufre dans la cuticule

1° Soufre inorganique

Il apparaît que le soufre inorganique sous forme de sulfate de sodium est uniformément incorporé à la cuticule, avant et après la mue. Seuls, les processus du condyle ne sont par radioactifs avant la mue, tandis que les épines tibiales le sont au contraire très fortement après la mue, et même en période de repos de la cuticule.

2° Soufre organique

La cuticule devient là aussi uniformément radioactive. Il est remarquable cependant que la méthionine provoque une radioactivité extrêmement forte lorsque l'injection est faite avant la mue, beaucoup plus intense que celle que l'on obtient dans les mêmes circonstances avec la cystine. Les deux substances sont - contrairement au soufre inorganique - présentes dans le processus du condyle avant comme après la mue. Les épines tibiales ne présentent pas ici d'activité particulièrement intense.

Il ressort de ces résultats que le soufre est souvent incorporé dans la cuticule, mais avec des modalités variées.

Le fait le plus frappant est la très forte radioactivité des protéines cuticulaires dans le cas d'injections de méthionine qui doit être l'acide aminé soufré essentiel pour cette formation.

Des différences entre soufre inorganique et soufre organique se font jour: au niveau des processus du condyle, seul le soufre organique est incorporé, de façon d'ailleurs importante, tandis que pour les épines tibiales, c'est le soufre inorganique qui paraît le plus utilisé, et cela même en période de repos.

CONCLUSIONS

Il faut tout d'abord souligner l'incorporation de soufre dans la cuticule au repos alors que la tyrosine et le tryptophane ne provoque pratiquement pas de rayonnement. Sauf dans le cas des épines du tibia, cette incorporation reste faible; on doit néanmoins en tenir compte dans toutes les expériences de ce type. L'interprétation n'en est pas aisée. La cuticule, durcie et pigmentée, est théoriquement inerte. En réalité, elle reste en relation avec l'hypoderme vivant sous-jacent qui conserve des prolongements cytoplasmiques dans les innombrables canalicules de la cuticule - relation qui reste à étudier.

Depuis longtemps la présence de soufre est connue par analyse chimique dans la cuticule et la possibilité de réactions de groupements sulfhydrilés des protéines cuticulaires avec des quinones est envisagée [3, 4]. Cette hypothèse n'est pas incompatible avec les résultats obtenus ici par autoradiographie. Comparés à ceux obtenus avec la tyrosine, ils attestent

que la présence de pigments issus de cet acide aminé n'est pas liée à une richesse différentielle en soufre ni que le soufre soit à l'origine des variations de coloration, l'incorporation étant ici la même dans une région pigmentée en noire ou une zone colorée en jaune.

Par contre, l'attention est attirée par le fait que seul le soufre organique est intégré notablement dans les processus du condyle dont on connaît l'accélération du durcissement et de la pigmentation et l'intégration également accélérée de la tyrosine. On peut peut-être évoquer ici les couplages obtenus in vitro entre dérivés quinoniques de la tyrosine et des thiols [5] pouvant donner naissance à des pigments sans que l'état hormonal de l'animal ne permette la libération des enzymes conditionnant l'évolution générale de la cuticule (voir le rôle de l'Ecdyson dans le déclenchement de l'activité décarboxylasique [6]).

Enfin, le fait que le soufre inorganique est incorporé à des structures organiques complexes telle que la cuticule amène à poser le problème de la conversion chez Gryllus du soufre inorganique en soufre organique, démontré chez quelques autres espèces [7]. Cet insecte possède en outre un segment intestinal à micro-organismes lequel, d'après MARTOJA [8], incorpore le soufre inorganique injecté dans la cavité générale, et peut jouer un rôle ici.

En conclusion, on peut dire que les pigments cuticulaires ont tous leur origine dans la tyrosine incorporée après la mue; le soufre est présent dans la cuticule de façon régulière, mais ne paraît pas jouer de rôle spécifique dans la pigmentation.

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DISCUSSION

C.E. SEKERIS: The autoradiographical method that you use is very interesting. In Munich, Frau Dr. Schlossberger is using similar techniques to study the incorporation of labelled tyrosine and tryptophane in the wild and albino strains of the grasshopper Schistocerca gregaria.

It would be interesting to use this technique for the incorporation in the cuticle not only of tyrosine but also of its metabolites, acids as well as dopamine in the different developmental stages. This could give some insight into the problems of sclerotization and pigmentation, especially in insects where these two processes are clearly separated.

Concerning your autoradiograms after injection of S-labelled compounds, is it possible that these substances are merely being inactivated and excreted in this way, as cuticulum?

S. FUZEAU-BRAESCH: Yes, that is certainly a possibility.

W. KLOFT: Mme Fuzeau-Braesch's method helps one to understand the mechanism of moulting in hemi-metabolic insects. The incorporation of the labelled substance in the femur condyle is most interesting from this point of view, and I am most grateful to her.

E. HORBER: I should like to mention that with certain Diptera, as Oscinis pumilionis or Meromyza americana, which develop in graminaceous plants, the phenomenon of sexual dimorphism has been observed in addition to very pronounced seasonal dimorphism. The males showed more melanine than the females, and the hibernating flies more than the generations produced during the summer. Perhaps these species could be considered in the studies as well as the Orthoptera which you have mentioned.

S. FUZEAU-BRAESCH: Thank you very much, that might be useful.

J. R. OGLE: I should like to say something with regard to your observations on the appearance of S^{35} from methionine an cystine into the cuticle. If, as Dr. Sekeris suggested, this could be caused by excretion into the cuticle, do you think that, in fact, it might be due to the deposition of the non-metabolized d-isomers, rather than a genuine incorporation of the sulphur from the l-isomer? Would it not be preferable to use the l-isomers of these labelled amino-acids?

S. FUZEAU-BRAESCH: Yes, this ought to be done. The substance we had at the time of the experiments was a mixture, not the pure l-isomer.

V.

RADIATION STUDIES: PRINCIPLES AND APPLICATION OF THE STERILE-MALE TECHNIQUE

EFFECTS OF IONIZING RADIATION ON INSECTS AND OTHER ARTHROPODS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF IONIZING RADIATION ON INSECTS AND OTHER ARTHROPODS. Research into the possible application of the radiation sterilization method of population suppression is now under way on a number of insects that attack man, animals and a variety of crops. These exploratory investigations have shown that ionizing irradiation will induce sterility but there is considerable variation in the amounts needed. The research also suggests that radiation damage may in some cases prevent application of the method to some insects. A frequent obstacle that must be overcome is lack of practical mass-rearing methods. Some insects also appear to be so abundant that the use of the technique may not be feasible without first processing the infested area with other control measures to bring wild populations within reach. Despite these difficulties, when conditions are favourable, few other approaches to the control of pests are so potentially rewarding. The radiation sterilization method may also be thought of as a possible means of delaying development of infestation until crops are harvested.

The present paper reports on the influence of gamma radiation on the reproductive potential, sexual aggressiveness, vigour and longevity of the oriental fruit fly, Dacus dorsalis Hendel, the melon fly, Dacus cucurbitae Coq., the Mediterranean fruit fly, Ceratitis capitata Wied., the Mexican fruit fly, Anastrepha ludens Loew, and Anopheles quadrimaculatus Say, and results of practical field trials of the sterile-male release method of population suppression. Progress in the campaign to eradicate the screw-worm, Cochliomyia hominivora Coql., in the United States and in studies to develop vigorous genetically marked strains that will permit ready identifications of released sterile flies is reviewed. Results of irradiation research on six additional species that infest fruit, vegetable, field and forest crops, three that attack livestock, three that largely affect man, the effects of irradiation on the scorpion, Centruroides limpidus Karsch, and the Lone-Star tick, Amblyomma americanum L., and ionizing radiation as a possible quarantine treatment for fruits and vegetables infested with fruit flies and mangoes infested with the mango weevil, Sternochetus mangiferae Fabricius, are also discussed.

EFFET DES RAYONNEMENTS IONISANTS SUR LES INSECTES ET AUTRES ARTHROPODES. Des recherches sur la possibilité d'appliquer la méthode de stérilization par les rayonnements pour l'éradication de populations d'insectes sont actuellement en cours pour un certain nombre d'insectes nuisibles à l'homme, aux animaux et à diverses cultures. Il ressort de ces recherches préliminaires que les rayonnements ionisants provoquent la stérilité, mais que les doses nécessaires varient considérablement. Ces recherches donnent aussi à penser que les dommages radioinduits sont tels qu'il ne serait pas opportun d'appliquer cette méthode à toutes les espèces d'insectes. Une difficulté à laquelle on se heurte fréquemment est l'absence de méthodes pratiques d'élevage en masse. D'autre part, certaines populations d'insectes sont tellement nombreuses qu'il peut être impossible de recourir à cette méthode sans avoir au préalable appliqué dans la région infestée d'autres méthodes de lutte de manière à pouvoir atteindre les populations naturelles. Malgré ces difficultés, si les conditions sont favorables, il existe peu d'autres méthodes qui puissent donner d'aussi bons résultats. On peut aussi envisager de recourir à la méthode de stérilisation par les rayonnements pour retarder la pullulation des insectes jusqu'à la moisson des récoltes.

Le mémoire rend compte de l'influence des rayons gamma sur la capacité de reproduction, l'agressivité sexuelle, la vigueur et la longévité de divers insectes: mouche orientale des fruits Dacus dorsalis Hendel, mouche du melon Dacus cucurbitae Coq., mouche méditerranéenne des fruits Ceratitis capitata Wied, mouche mexicaine des fruits Anastrepha ludens Loew et Anopheles quadrimaculatus Say; il donne aussi les résultats d'expériences réelles de lâcher de mâles stériles pour l'éradication des insectes. L'auteur analyse les résultats obtenus dans la campagne d'éradication de la lucilie bouchère Cochliomyia hominivorax Coql. aux Etats-Unis, ainsi que dans des études visant à produire des souches vigoureuses, marquées génétiquement en vue d'identifier

facilement les mouches stériles après leur lâcher. Il étudie aussi les résultats de recherches sur différents sujets: effets des rayonnements sur six autres espèces nuisibles aux fruits, aux légumes, aux cultures et aux forêts, sur trois espèces qui s'attaquent au cheptel et sur trois espèces qui sont particulièrement nuisibles à l'homme; effets des rayonnements sur le scorpion Centruroides limpidus Karsch et sur la tique Amblyomma americanium L.; possibilité d'utiliser les rayonnements ionisants pour traiter - comme mesure de quarantaine - les fruits et légumes attaqués par les mouches des fruits et les mangues attaquées par le charançon de la mangue Sternochetus mangiferae Fabricius.

ВОЗДЕЙСТВИЕ ИОНИЗИРУЮЩЕЙ РАДИАЦИИ НА НАСЕКОМЫХ И ДРУГИХ ЧЛЕНИСТОНОГИХ. В настоящее время проводятся исследования возможности применения метода стерилизации посредством облучения для уничтожения популяций целого ряда насекомых, поражающих человека, животных и различные культуры. Эти предварительные исследования показали, что ионизирующее облучение приводит к стерилизации, но что для этой цели требуются чрезвычайно разнообразные дозы. Оказалось, что в некоторых случаях радиационные повреждения могут исключить возможность применения этого метода у некоторых насекомых. Препятствие, которое зачастую приходится преодолевать, заключается в отсутствии практических методов разведения насекомых в массовых количествах. С другой стороны, некоторые виды насекомых являются настолько многочисленными, что применение этого метода может оказаться неосуществимым без предварительной обработки зараженного района другими средствами истребления для привлечения на доступное расстояние популяций насекомых. Несмотря на эти затруднения, при благоприятных обстоятельствах вряд ли какие-либо другие методы истребления вредных насекомых представляются столь же перспективными. Метод стерилизации при помощи радиации может служить способом отдаления инвазии до окончания сбора урожая.

В докладе сообщается о воздействии гамма-излучения на потенциал размножения, половую агрессивность, силу и живучесть восточной плодовой мухи Dacus dorsalis (Hendel), дынной мухи Dacus cucurbitae (Coq.), средиземноморской плодовой мухи Ceratitis capitata (Wied.), мексиканской плодовой мухи Anastrepha ludens (Loew) и малярийного комара Anopheles quadrimaculatus (Say); в докладе приводятся также результаты практических испытаний выпуска стерилизованных самцов на опытное поле для истребления популяций. Дается описание дальнейшего хода кампании по истреблению бычьего овода Cochliomyia hominivorax (Coqrl.) в Соединенных Штатах Америки и изучения разведения сильных, генетически маркированных особей, которые позволят легко выявлять выпущенных на волю стерилизованных оводов. Обсуждаются также результаты исследований по облучению 6 дополнительных видов вредителей, поражающих фрукты, овощи, полевые и лесные культуры, 3 видов, поражающих скот, и 3 видов, нападающих главным образом на человека, а также воздействие облучения на скорпионов Centruroides limpidus (Karsch), одновездчатого клеща Amblyomma americanum (L.); далее обсуждается возможность использования ионизирующего излучения в качестве возможного гарантийного средства для дезинфестации овощей и фруктов, пораженных плодовой мухой, а также манго, пораженного манговым долгоносиком Sternochetus mangiferae (Fabricius).

EFECTOS DE LAS RADIACIONES IONIZANTES SOBRE LOS INSECTOS Y OTROS ARTRÓPODOS. Actualmente se estudia la posibilidad de recurrir a la radioesterilización para exterminar a diversos insectos que atacan al hombre, a los animales y a las plantas. Las primeras investigaciones han demostrado que las radiaciones ionizantes producen esterilidad, y que las dosis necesarias varían considerablemente. También hacen pensar que los daños producidos por las radiaciones pudieran en algunos casos impedir que se aplique el método a ciertos insectos. Con frecuencia, el obstáculo principal es la falta de métodos de cría de los insectos en gran escala. En otros casos, el insecto se da con tal abundancia que no es posible aplicar esta técnica sin antes tratar por otros medios la zona infestada a fin de reducir el número de insectos. A pesar de todas las dificultades, el método de la radioesterilización es uno de los mejores, cuando las condiciones son favorables. También se puede utilizar para retrasar el desarrollo de la plaga hasta después de la cosecha.

La memoria informa sobre la influencia de las radiaciones gamma en la capacidad de reproducción, los instintos sexuales, el vigor y la longevidad de la mosca oriental de la fruta, Dacus dorsalis Hendel, la mosca del melón, Dacus cucurbitae Coq., la mosca mediterránea, Ceratitis capitata Wied., la mosca de la fruta mejicana, Anastrepha ludens Loew, y el Anopheles quadrimaculatus Say, e indica los resultados de la campaña de exterminio obtenidos en la práctica liberando machos estériles. Revisa también los progresos realizados en los Estados Unidos en la campaña para exterminar la Cochliomya hominivorax Cqrl., y en los estudios para desarrollar cepas vigorosas, marcadas genéticamente, que permitan identificar con facilidad las moscas estériles liberadas. Se discuten también los resultados de las investigaciones sobre la irradiación de

otras seis especies que atacan a frutas, verduras y otros cultivos agrícolas y forestales, otras tres que atacan al ganado, y tres más que atacan principalmente al hombre. Se trata asimismo de la irradiación del escorpión, Centruroides limpidus Karsch y del arácnido Amblyomma americanum L., y de la posibilidad de emplear radiaciones ionizantes como tratamiento de cuarentena para las frutas y verduras infestadas con la mosca de la fruta y para los mangos infestados con el gorgojo Sternochetus mangiferae Fabricius.

1. INTRODUCTION

The eradication of the screw-worm (Cochliomyia hominivorax Coquerel) from the island of Curaçao by the release of flies sterilized with gamma radiation [3] prompted worldwide studies to explore other applications for this unique method. The recent progress in radiation-sterilization research now under way at laboratories of the United States Department of Agriculture is reviewed in this paper. Limited reference is also made to information from other sources.

The most important requirements for successful application of the sterile-male release method are ability to mass-produce and sterilize the pest without serious damage to mating competitiveness or other behaviour, and sustained releases of sterile males at overflooding rates that will void the reproductive potential of the wild population [19].

2. RADIATION STERILIZATION AND INFLUENCE ON BEHAVIOUR

Three destructive tropical fruit flies, the oriental fruit fly (Dacus dorsalis Hendel), the melon fly (Dacus cucurbitae Coquillett), and the Mediterranean fruit fly (Ceratitis capitata Weidemann) have been studied intensively in Hawaii [6, 32, 33, 36]. The minimum dosage required to prevent egg-laying and render the males incapable of fertilizing females is about 10 000 r when the radiation is applied to late-stage pupae, with no significant difference among the three species. At sexual maturity the testes in treated individuals are full of sperm, but no further production of sperm occurs and few remain after 30-50 d. In cage tests with irradiated males in competition with normal males, the irradiated males were less than half as effective as normal males. In large-cage tests, however, ratios of irradiated to normal flies ranging from 7.5:1 to 20:1 suppressed reproductive potential by as much as 92% in two generations.

In studies with the Mexican fruit fly (Anastrepha ludens Loew) 12-d-old pupae were sterile after exposure to 5000 r of gamma radiation [30]. Both sexes were permanently sterilized and no eggs were produced by females. Emergence and longevity appeared to be normal. The sexual aggressiveness of young treated males was not affected. However, 30- to 39-d-old sterilized males were much less effective than normal males in competitive mating trials. More recent studies [29] indicated that, after irradiated males had mated with virgin females five or six times, few motile sperm remained in the testes and few or no sperm could be found in the spermatheca. In cage tests with ratios of sterile Mexican fruit flies to normal flies ranging from 1:1 to 50:1, fertility was greatly reduced, almost completely so at the 50:1 ratio.

In tests to determine sensitivity of the Mexican fruit fly to dose-rate of gamma radiation [30], 12-d-old pupae were irradiated at rates ranging from 10 to 90 r/min. At a dosage of 2000 r females deposited fewer eggs and males became less fertile as the dose-rate increased. No difference in emergence or longevity was observed in adults developing from pupae that were treated with 5000 r at 42.8 r/min or at 2695 r/min and there was no effect on sterility when treatment was administered in one dose of 5000 r or in two doses of 2500 r with a 24-h interval between radiation periods [29]. In sterilization of C. hominivorax it was found generally that multiple irradiation was more harmful than a single dose [11].

Mexican fruit-fly pupae have also been irradiated in atmospheres of nitrogen flowing at 3 l/min and of oxygen flowing at 5 l/min [29]. In nitrogen irradiation at 7000 r, a dosage of 2000 r higher than that required for sterilization in normal air, there was no effect on egg production or viability as had been noted in tests with C. hominivorax [20]. Females from pupae irradiated at 7000 r in oxygen laid some viable eggs, a result difficult to explain, since high oxygen tension normally increases the radiosensitivity of insect cells [10].

The influence of temperature on effects of radiation on insects has not been investigated extensively, but according to some investigators, temperature may be a critical factor [20], or at least have some significance [10]. Irradiation of dermestid larvae at 5000 rad before treatment with heat at 47°C interrupted the life-cycle, an effect that was not accomplished by either treatment alone [18].

Anopheles quadrimaculatus Say can be sterilized with 8 865 to 12 900 r of gamma radiation applied to the pupal or adult stage [9]. Irradiation of the pupae did not affect adult emergence, but there was considerable mortality of irradiated mosquitoes for the first 3 d after emergence. No gross differences were found between normal and sterile males with regard to sexual development and mating behaviour [36].

Aëdes aegypti L. may be sterilized with 11 000 to 18 000 r of gamma radiation [27]. In laboratory tests the production of viable eggs was greatly reduced when normal females were caged with normal and sterile males at a ratio of the latter of 1:20 [26].

Studies conducted on Drosophila melanogaster Meigen at Beltsville, Maryland, in 1962 indicated that untreated females mated with males exposed to 5 kr of gamma radiation in the larval and 20 kr in the pupal or adult stage deposited the normal number of eggs, none of which hatched. The longevity of males or females exposed in the pupal or adult stage was not affected by the radiation treatment, but males and females irradiated in the larval stage were shorter-lived than untreated insects. Sterile males confined with normal males and females reduced the number of progeny [15]. Immediately after treatment, 3- to 4-d-old males exposed to 16 kr did not mate as readily or as many times with virgin females as untreated males or males exposed to 8 kr. Males exposed to 16 kr recovered within 24 hours and their mating frequency and behaviour were normal [16].

Adults of the Mexican bean beetle (Epilachna varivestis Mulsant) were sterilized by exposure to 10 or 20 kr of gamma radiation. Pupae were more susceptible than adults. Dosages ranging from 1 to 16 kr resulted in sterile females, whereas males were sterilized at 4 to 16 kr. Larvae were more

susceptible to direct radiation effects than pupae and pupae were more susceptible than adults [17].

In studies with the European corn borer (Ostrinia nubilalis Hübner) at Ankeny, Iowa, untreated virgin female moths mated with male moths treated at 1 d after emergence with 32 kr of X-rays laid eggs of which only 1% hatched. Irradiated males competed equally with untreated males for females. Moths caged together at a ratio of 8 irradiated males to 4 untreated males to 8 untreated females resulted in 39.4% hatch of eggs. The survival of irradiated males compared favourably with that of untreated males under laboratory conditions. Female pupae were more susceptible to irradiation than males and younger pupae were more susceptible than older pupae [35].

At Brownsville, Texas, males of the pink bollworm (Pectinophora gossypiella Saunders) from irradiated 7-d-old pupae mated with untreated females resulted in complete sterility when irradiated at 60 kr, and at 30-60 kr no more than 1.7% of the eggs hatched. Exposure at 40 kr was sufficient to sterilize females. The longevity of males from 7-d-old pupae treated with 35-90 kr was significantly shorter than that of controls [28].

Irradiation of virgin males of the boll weevil (Anthonomus grandis Boheman) at 10 kr resulted in transient sterility, whereas 15 kr produced permanent sterility; however, these doses caused very rapid mortality of both sexes [8].

At New Haven, Connecticut, treatment of the gypsy moth (Porthetria dispar L.) with gamma radiation showed that males can be successfully sterilized and that the optimum dosage is 20 kr for 9- to 11-d-old pupae. The competitiveness and efficiency of treated males under natural conditions remain to be determined [13].

In an exploratory study with the stable fly (Stomoxys calcitrans L.) at Kerrville, Texas, 5 kr of gamma radiation to the late pupal stage resulted in complete sterility of both sexes. Although longevity was not measured, it appeared that there was very little effect from this dosage [14].

In laboratory studies conducted at Corvallis, Oregon, both sexes of the hornfly (Haematobia irritans L.) were sterilized by 5 kr of gamma radiation and longevity was unaffected. When flies from irradiated and untreated pupae were allowed to emerge together in screened cages, the eggs obtained were less viable than those in the control in every test [22].

Studies also at Corvallis, Oregon, with Culex tarsalis Colquillett showed that the male was sterilized at 15 kr and the female at 5 kr, with very little effect on longevity. Competitiveness was reported as good, but the study is still incomplete [7].

In exploratory tests conducted at Kerrville, Texas, with the Lone Star tick (Amblyomma americanum L.), females were sterilized at relatively low dosages, 500 to 2500 r. Higher dosages were required for males. Newly emerged adults were more easily sterilized than nymphs [12].

The scorpion (Centruroides limpidus Karsch) was treated with radiation in 4 lots of 15. At 4500 r there was little or no effect and one scorpion lived 105 d after treatment. At 8000 r the scorpions remained motionless and refused food; however, one lived for 33 d after treatment. At 10 000 r all were dead after 17 d. At 11 500 r all scorpions were dead at 7 d. In a control of 100 some individuals lived 6 months [24].

3. INDUCED STERILITY IN INSECT CONTROL AND ERADICATION

An economical method of rearing insects in large numbers is a requirement for utilization of the sterile-male technique [19, 36]. The availability of such methods for mass-rearing several species of tropical fruit flies made it possible to initiate field tests after appropriate radiation sterilization dosages were determined. A field test of the effect of releases of sterile Mediterranean fruit flies was undertaken in May, 1959, in an isolated 12-mile^2 host-fruit area on the island of Hawaii at an altitude of 3600-6000 ft [33]. Nearly 2 months were required for each generation of Medfly at this elevation, where temperatures seldom exceeded 60°F in the shade for more than 2-6 h/d. The test was terminated 1 July 1960, after a total of 18.7 million flies irradiated at 10 000 r in the pupal stage had been released. A mean reduction of approximately 90% in the population of Medflies was obtained.

In December 1960, releases of sterile oriental fruit flies were started on the 33-mile^2 island of Rota, an isolated island in the Western Pacific, following a year's trapping to determine the normal population density and trends. In this experiment the flies were reared to the pupal stage and irradiated in Hawaii and then shipped by air to Guam for aerial and ground releases of adults on Rota. Progress was evaluated by means of trap catches for adults and the holding of fruit samples for emergence of larval populations. The sterile flies were released weekly by air along flight lines $\frac{1}{2}$ mile apart running lengthwise to the island and from ground emergence cages placed at strategic locations [33]. This oriental fruit fly experiment was terminated in June, 1962, after 21 months, during which an estimated 410 million sterile flies were released on Rota. The target overflooding ratio of 10 to 1 of sterile to normal males was never achieved on Rota on an island-wide, sustained basis. Even though its objective was not achieved, the test nevertheless provided a wealth of valuable experience and knowledge of factors that may influence effective application of the method to an unusually abundant insect [34].

In September, 1962, releases of sterile melon flies, a much less abundant species than the oriental fruit fly on Rota, were initiated after the native population had first been reduced with poison bait sprays applied around farms and other host areas. Ratios higher than 30:1 of sterile-to-normal flies were quickly attained and complete suppression of the melon-fly population is anticipated [34]. In several recent weeks there has been no evidence of infestation in preferred hosts.

Studies involving releases of Mexican fruit flies sterilized with gamma radiation have been conducted at two locations in Mexico. In 1961, at one location, a ratio of 66:1 gravid-normal flies was achieved and the larval population in mangoes reduced to an unusually low level of 0.37 per lb. In 1962, with a high natural population, a ratio of 8:1 was insufficient to provide population suppression [29].

A. quadrimaculatus males sterilized in the pupal stage were released into natural populations of quadrimaculatus at two localities in Florida [37]. During an 11-month period 328 900 males were liberated at 9 to 10 release points on a small semi-isolated island in Lake Okeechobee, and 104 700 were liberated during an 11-wk period at two release points within an extensive

breeding area in a swamp at the south end of Lake Panasoffkee, Sumter County. It was concluded that the release of sterile males may have influenced the abundance of quadrimaculatus in the Okeechobee experiment when the natural population was in a seasonal decline, but such a release had no effect when conditions were more favourable for reproduction and development. The release of sterile males in the Lake Panasoffkee area did not conclusively demonstrate any induced sterility in wild females. Further studies on the biology and behaviour of this species are being undertaken.

A sterile release test with A. aegypti was made in two areas in the vicinity of Pensacola, Florida [27], during which 3 912 000 sterile pupae were received for liberation in 1960 (96% males) and 6 708 600 for liberation in 1961 (97% males). It was concluded from the results of the experiment that the adaptation of the sterile-male method for mosquito control will require additional biological investigations, especially of the dispersion of males under field conditions.

A co-operative programme to eradicate the screw-worm throughout the south-eastern United States by means of releases of sterile flies was undertaken in 1958 by the United States Department of Agriculture and the State of Florida [23, 36]. An average of 50 million screw-worm flies were reared, sterilized and released per week to achieve eradication in less than 2 yr in the southern United States east of the Mississippi River.

Early in 1962, a co-operative programme was undertaken by the United States Department of Agriculture, the Southwest Animal Health Research Foundation, the Texas Animal Health Commission and other public and private groups in several States to utilize releases of sterile flies to eradicate the screw-worm from the south-western United States and to maintain a barrier against re-infestation from Mexico. The rate of sterile fly releases in the areas in Texas where the insect is normally able to overwinter had reached over 113 million/wk by early February 1963.

Inability to identify released insects positively is a serious handicap in evaluation. A genetic strain of white-marked oriental fruit flies which had been found to occur naturally in 0.5% of the wild Rota population was developed in Hawaii. In comparative tests, the white-marked strain has appeared to be equal in behaviour and reproductive capacity and compatible with normal yellow laboratory flies. The white-marked oriental fruit fly strain was used in the Rota test beginning with the releases made in August 1961 [33]. Mexican fruit-fly rearing stocks are observed for easily detectable aberrations which might be developed as genetic markers and F_1 and F_2 progeny or males irradiated with 1500 r and mated with untreated females are scanned. A female with a dark V on the scutellum was obtained from a total of 30 000 F_2 -generation flies, but death occurred before sexual maturity [29].

In the search for mutants of the screw-worm useful for marker stocks, the flies scanned are from three sources, untreated laboratory-reared flies, progeny from irradiated normal parents and progeny from irradiated flies inbred for two generations. A number of mutants have been observed but none has been fully compatible and competitive with wild flies [21].

4. IONIZING RADIATION AS A COMMODITY TREATMENT

The development of irradiation methods for treatment of fruit and other commodities susceptible to infestation by fruit flies and other insects may offer a means of quarantine treatment that is more effective and less undesirable than fumigation or other treatments.

The first successful test with ionizing radiation to free a commodity from insects apparently was with tobacco infested with the tobacco or cigarette beetle (Lasioderma serricorne F.) in 1916 [31]. In the Medfly eradication campaign in Florida in 1929, a few exposed larvae and larvae within fruit were treated with X-rays. It was concluded that the exposures were insufficient to kill larvae, either exposed or within fruit [25].

Studies in Honolulu, Hawaii, indicated that 300 kr was not immediately lethal to the immature stages of the oriental fruit fly. However, the development of irradiated eggs and larvae to the adult stage was prevented by dosages of 7.5 and 15 kr [1]. The results of further investigations with the immature stages of the oriental fruit fly, the melon fly and the Medfly suggested that comparatively low dosages in the range 15 - 20 kr would provide an effective commodity treatment for fruit-fly-infested fresh fruits and vegetables [2].

Larvae of the mango weevil (Sternochetus mangiferae F.) in mango seeds treated with 5 kr of gamma radiation failed to develop to adults and were prevented from forming pupae by an exposure of 12.5 kr. Sexually mature adults irradiated in shell vials were sterilized by 5 kr and sexually immature adults treated within the bare mango seed were sterilized by 15 kr [2].

Grapefruit infested with eggs and first-stage larvae of the Mexican fruit fly exposed to 5 krad showed no insects or insect damage when dissected. Fruit infested with mature larvae produced numerous pupae; however, no adult flies emerged from pupae from fruit treated at 5 krad or higher [5].

Mangoes infested with the Mexican fruit fly were treated with gamma radiation at dosages ranging from 500 to 5000 r at the rate of 1500 r/min. Flies surviving the 1000-r dose did not oviposit and only one malformed male survived at each of the 2000 and 2500-r treatments and no adults emerged at 3000 r or above. In further studies, X-ray radiation appeared to be as lethal to the Mexican fruit fly as gamma radiation [4].

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DISCUSSION

M. S. QURAISHI: How were the male mosquitoes separated from the female mosquitoes for the release of males?

W. E. STONE: I regret I cannot tell you that because I am simply quoting what was done by Dr. Schmidt and Dr. Morlan and their associates. Dr. Schmidt could undoubtedly tell you.

M. S. QURAISHI: We have found that the mechanical separator described by McCray and Morlan is quite efficient in separating male and female larvae of Anopheles stephensi, but of course you do not get 100% separation. Perhaps Dr. Schmidt could throw some more light on this.

C.H. SCHMIDT: In the experiments on the release of sterile males for the control of Anopheles quadrimaculatus (ref. [37] to the paper), the sex separation was carried out in the adult stage. Within 24-48 h after the adults emerged, they were inactivated in a cold room (35°-40°F), the females removed and the males transferred to cages for release in the test area. Females were not released, since their bites would have caused annoyance and their presence in the resting stations in the test area would have introduced errors in the weekly adult counts. Before we undertake a large-scale test we shall have to devise some better means of separating the sexes. This was much too tedious.

J.W. MILES: Dr. Schmidt, when you irradiated these insects, the irradiation was done on the pupae, was it not, the adults being separated afterwards? In previous work, by McCray et al. (ref. [26] to the paper), I believe they irradiated the male pupae after separation.

C.H. SCHMIDT: Yes, we irradiated quadrimaculatus pupae which were not more than 24-h old.

P. NARDON: You said, Dr. Stone, that you cultured the Mexican fruit fly in order to see whether any aberrations appeared. Personally, I think aberrations are bound to appear. I have observed them with Sitophilus grain weevils, and the paper I am to present tomorrow * has some bearing on this matter. But could you, in particular, give us some indication as to the likelihood of reduced fertility? In my opinion it is particularly on the physiological side that one may expect important changes.

W.E. STONE: The Mexican fruit flies were exposed to 1500 r, and this was sufficient to reduce fertility somewhat; we believe, however, that fertility can be restored if a satisfactory mutant is obtained. As far as other aberrations are concerned all I can say is that out of some 20 000 irradiated flies we found an inverted "V" on the scutellum in one female, but unfortunately she died before she could lay eggs. That often happens, the geneticists tell me, in working with stocks that have been irradiated.

A. SÜSS: Have you found any relation between temperature and sterilization dose?

W.E. STONE: All our sterilization has been done at about 25-27°C — more or less at room temperature. We have not, ourselves, investigated the effect of temperature in relation to irradiation, but work done by Dr. Nair on this question was referred to by Horne and Brownell in the paper cited as ref. [18] to my paper. The paper by Nair and Rahalkar on their work with the Khapra beetle † contains further interesting information on this subject. I think that D.J. Jeffreys has also done some work on the relation of temperature to irradiation in the case of Sitophilus granarius.

R. von BORSTEL: I think the United States Department of Agriculture is to be congratulated on the marvellous work done on eradication of insect pests by the male irradiation method. I would like to know why the particular dose of 1500 r was selected for the screening of mutations in Anastrepha sperm.

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* LAVIOLETTE, P. et NARDON, P., Influence de l'irradiation sur les adultes de Sitophilus sasakii takahashi (Curculionidae) et leurs descendants, these Proceedings.

† NAIR, K.K. and RAHALKAR, G.W., Studies on the effects of gamma radiation on the different development stages of the Khapra beetle, Trogoderma granarium Everts., these Proceedings.

W.E. STONE: The 1500-r exposure was selected for Anastrepha ludens arbitrarily, as it is between 1000 r, which has little effect on oviposition and hatch, and 2000 r, which is considered a critical dosage.

R. von BORSTEL: Who is carrying on that work?

W.E. STONE: LaChance. A very full account of it appears in the article cited as ref. [21] to my paper.

M. FRIED: Have you any more recent information as to the results of the melon-fly tests on the island of Rota?

W.E. STONE: Nothing more than what is said in the paper. I would like to mention that the main problem in all our eradication work by the sterile-male technique is the problem of ensuring sufficient isolation. You may think you have pretty good isolation but then the insects begin to come in: Steiner and his associates in Hawaii have found on the island of Rota marked melon flies that were released on the island of Guam 40 miles away; in Mexico, we are facing a similar situation with the Mexican fruit fly. You would think that in a country as large as Mexico we would be able to find isolated places to carry out our experiments, but it is almost impossible. Now, Greece, I should say, would be situated ideally, with its many islands, some of them quite far apart. This problem of isolation is a tremendous problem: in the south-western United States, for a week or two, there were no screw-worm cases, and then all of a sudden they began to reappear.

M.S. QURAISHI: Talking of sterilizing insects, I was wondering if Dr. Stone, or any other scientist present, would like to comment on the chemical sterilants regarding which quite a few papers have appeared in recent literature.

W.E. STONE: In Mexico, most of our sterile release work at the present time is being done with flies sterilized in the pupal stage with TEPA. We have a paper in press at the present time, which should appear in six to eight months in the Journal of Economic Entomology, reporting on the results of release of flies sterilized with TEPA in a ten-acre mango grove in Mexico. If we had had complete isolation I think our results would have been very much better, but they were so good that for the first time in history at that particular grove about 90% of the crop was harvested as acceptable fruit; in the control area, about a mile away, all the fruit was gone two or three weeks previously. This product TEPA, which we selected out of some 1500 for development at the present time, is a most promising material, though it should be handled with care and we make sure that our workers use gloves, and when the pupae are dipped we have good extractor fans operating in the room. We also have chemosterilant stations where we are testing, but we now have them under guard night and day.

K. van ASPEREN: Do you know of any experiments on the development of resistance to ionizing radiation in insects?

W.E. STONE: No, I do not. There are workers here much more capable of answering that question than I.

R. von BORSTEL: I know of only one large-scale experiment in insects designed to select for mutations conferring resistance to radiation. This was an experiment carried out by Bruce Wallace on populations of Drosophila melanogaster under chronic radiation conditions at such a high level that population decline was continuous. Every few generations it was necessary

to halt the irradiation, build up the population, and begin the irradiation again. Radiation-resistant flies were not obtained.

R. DELATTRE: The insects mentioned in your paper, Dr. Stone, are all of the holometabolous type: dipterous, lepidopterous, etc. Is there any inherent difficulty in applying this method to hemimetabolous insects?

W.E. STONE: I have never been associated with any studies in which hemimetabolous insects have been irradiated. The only cases I know of are the Lone Star tick, which is not strictly speaking an insect, and the scorpion in Mexico. I am sure that other work has been done, but I do not know who has done it.

H. HUQUE: Do you know whether any irradiation work is being done by the United States Department of Agriculture on plant quarantine?

W.E. STONE: I do not know of any such work in the United States as such, within the continental limits. In the work we did for the United States Department of Agriculture in Mexico with the Mexican fruit fly and the work I referred to with the Dacus, the Mediterranean fruit fly and the mango weevil, all of which was done in Hawaii, the idea of this work was in fact to develop a possible quarantine method. But I cannot tell you what, if anything, has been done in the United States itself.

ERADICATION OF WHITE GRUB (MELOLONTHA VULGARIS F.) BY THE STERILE - MALE TECHNIQUE

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Abstract — Résumé — Аннотация — Resumen

ERADICATION OF WHITE GRUB (MELOLONTHA VULGARIS F.) BY THE STERILE-MALE TECHNIQUE. Laboratory tests indicated that an X-ray dose of 3000 r was sufficient to induce sterility in male cockchafers. During two flight periods, sterilized males were released among a natural population in order to eliminate white grubs in a general farming region of north-western Switzerland. In 1950 an outbreak of this pest was reduced by a chemical treatment. Gradation had been watched during every flight from 1953 to 1962.

In 1959 five areas, each with a surface of about 30 ha, were selected to serve as:

(a) The treated area, where the males were captured, irradiated and released;

(b) The "bank," where cockchafers were collected and the males were irradiated for release in area a; and

(c) Control areas, where undisturbed gradation was observed.

The males were irradiated in a therapeutical X-ray unit. Irradiated males were hand-painted in order to estimate the ratio of sterilized males by means of the isotopic dilution technique.

In 1959, for the first treatment, about 6 l of sterilized males, representing about 50% of the male population, were released in (a).The white grub infestation sampled in grassland dropped thereafter to about two-thirds of that in the other areas. The reproduction rate was less than unity only in (a). A further reduction of the population in (a) to one-tenth of that in (b) and (c) was observed when the number of surviving cockchafers was estimated in 1962. The greatest mortality from 1959 to 1962 occurred in (a).

In 1962, for the second treatment, a total of 17 l of irradiated males was released in (a). At least 76% of the male population of (a) had been sterilized. The following sampling of the white grub population showed complete eradication in (a). Some reduction was also observed in (b) and (c) due to drought in the whole region.

It has been demonstrated that the sterile-male technique may successfully be applied to an insect pest in an area which is not strictly isolated geographically, the females of which mate several times and the breeding of which in large masses is not feasible because of the long breeding cycle.

ÉRADICATION DU VER BLANC (MELOLONTHA VULGARIS F.) PAR LA MÉTHODE DU LÂCHER DE MÂLES STÉRILES. Des essais en laboratoire ont montré qu'une dose de rayons X de 3000 r suffit pour stériliser les hannetons mâles. Des mâles stérilisés ont été lâchés, au cours de deux apparitions, dans une population naturelle en vue d'éliminer les vers blancs dans une région essentiellement agricole du nord-ouest de la Suisse. En 1950, une invasion de hannetons avait pu être enrayée à l'aide d'un traitement chimique. L'évolution ultérieure a été observée à chaque apparition, de 1953 à 1962.

En 1959, on a choisi cinq zones d'environ 30 hectares chacune, réparties en trois catégories:

a) Zone traitée, où les mâles étaient capturés, irradiés et relâchés;

b) Zone de réserve, où les hannetons étaient ramassés, les mâles irradiés et relâchés en a;

c) Zones témoins, où l'on observait l'évolution naturelle.

L'irradiation des mâles était effectuée dans un appareil de roentgenthérapie. Les mâles irradiés recevaient des marques peintes à la main, afin de pouvoir évaluer la proportion de mâles stériles par la méthode de dilution isotopique.

En 1959, année du premier traitement, on a relâché en a) environ 6 l de mâles stérilisés, ce qui représentait près de 50% de la population mâle. D'après les échantillons prélevés dans les prés, le nombre de vers blancs n'était plus par la suite que les deux tiers de leur nombre dans les autres zones. Le taux de reproduction n'était inférieur à l'unité que dans la zone a). En évaluant le nombre des hannetons survivants en 1962, on a constaté que la population de a) était tombée à un dixième de celle de b) et c). C'est en a) que la mortalité a été la plus élevée entre 1959 et 1962.

En 1962, année du deuxième traitement, on a relâché en a) 17 l de mâles irradiés. La population mâle de a) avait été stérilisée dans la proportion d'au moins 76%. Les échantillons prélevés par la suite ont montré que l'éradication des vers blancs en a) était absolue. On a également observé une certaine diminution des larves en b) et c), due à la sécheresse qui régnait dans toute la région.

Il a été démontré que la méthode du lâcher de mâles stériles peut être appliquée avec succès dans la lutte contre un insecte nuisible qui se trouve dans une région qui n'est pas strictement isolée du point de vue géographique, dont les femelles s'apparient plusieurs fois et dont l'élevage en masse est impossible en raison de la durée du cycle évolutif.

УНИЧТОЖЕНИЕ ЛИЧИНОК МАЙСКОГО ЖУКА (MELOLONTHA VULGARIS F.) МЕТОДОМ СТЕРИЛИЗАЦИИ МУЖСКИХ ОСОБЕЙ. Лабораторные испытания показывают, что доза 3000 р достаточна для стерилизации мужских особей майского жука. В течение двух вылетов стерилизованные самцы выпускались на волю среди необлученных популяций для ликвидации хрущей в районе с неспециализированным сельским хозяйством, расположенном в северо-западной Швейцарии. В 1950 году широкое распространение этих вредителей было предотвращено применением химикалий. Спаривания наблюдались при вылете с 1953 по 1962 год.

В 1959 году были отведены пять участков площадью 30 га, на которых создали:

А - экспериментальный участок, на котором мужские особи вылавливались, облучались и выпускались снова на волю;

Б - участок для разведения, где майские жуки собирались, их мужские особи облучались и выпускались на участок А;

В - контрольные участки, где наблюдались спаривания без внешнего вмешательства.

Самцы облучались на обычной терапевтической рентгеновской установке. Облученные самцы окрашивались вручную для определения пропорции стерилизованных самцов методом изотопного разведения.

В 1959 году в качестве первого опыта на участок А было выпущено около 6 л стерилизованных самцов, что составляло около 50% всей популяции самцов. Пробы, взятые с пастбищ, показали, что заражение хрущами сократилось приблизительно на 1/3 по сравнению с другими участками. Коэффициент размножения был меньше единицы только на участке А. Когда в 1962 году была произведена оценка числа выживших майских жуков, то на участке А было обнаружено дальнейшее сокращение популяции на 1/10 по сравнению с участками Б и В. Наибольшая смертность с 1959 до 1962 года наблюдалась на участке А.

В 1962 году для второго опыта на участке А было выпущено 17 л облученных самцов. По меньшей мере, 76% популяции самцов на участке А было стерилизовано. Последующее определение популяции хрущей показало их полное истребление на участке А. Некоторое сокращение наблюдалось также на участках Б и В в результате засухи в этом районе.

Было показано, что метод стерилизации самцов может с успехом применяться на участке без строгой географической изоляции для борьбы с насекомыми-вредителями, самки которых спариваются по нескольку раз в сезон и разведение которых в больших количествах неосуществимо ввиду значительной продолжительности цикла их выведения.

ERRADICACIÓN DE LA LARVA DEL ABEJORRO (MELOLONTHA VULGARIS F.) POR LA TÉCNICA DE LOS MACHOS ESTERILIZADOS. Ensayos realizados en laboratorio indicaron que para esterilizar a los abejorros machos basta con una dosis de rayos X de 3000 roentgens. Durante dos períodos de vuelo se soltaron machos esterilizados en una región agrícola del noroeste de Suiza con objeto de erradicar las larvas. En 1950 esta plaga había sido combatida con procedimientos químicos. Entre 1953 y 1962 se observó la actividad en todos los vuelos.

En 1959 se seleccionaron cinco zonas de 30 hectáreas cada una que sirvieron de:

a) Zona de tratamiento, en la que se capturaron, irradiaron y soltaron machos;

b) Zona de reserva, en la que se recogieron abejorros y se irradiaron machos que se soltaron en a);

c) Zonas de control, en las que se observó la actividad normal.

Los machos fueron irradiados con un aparato terapéutico de rayos X. Después de la irradiación se les puso una marca de color para poder calcular su porcentaje mediante la técnica de dilución isotópica.

En 1959, durante el primer tratamiento, se soltaron en la zona a unos 6 l de machos esterilizados, lo que representaba aproximadamente el 50% del total de machos de dicha zona. La cantidad de larvas recogidas en los prados después del tratamiento representó unos 2/3 de la correspondiente a las zonas de control. La razón de reproducción sólo fue inferior a la unidad en la zona a). Cuando se calculó, en 1962, el número de

abejorros supervivientes se observó que en la zona a había disminuido hasta 1/10 en comparación con las zonas b) y c). De 1959 a 1962 la cifra más elevada de mortalidad se observó en la zona a).

En 1962 se procedió en esta zona a un segundo tratamiento para el que se soltaron 17 1 de machos irradiados. Se había esterilizado el 76% por lo menos de los machos. El nuevo muestreo de larvas demostró que en la zona a) el exterminio había sido completo. Debido a la sequía que reinó en toda la región se observaron también disminuciones en las zonas b) y c).

Se ha demostrado que la técnica de los machos esterilizados puede aplicarse con éxito a insectos de una región que no esté estrictamente aislada desde el punto de vista geográfico, cuyas hembras copulen varias veces, y que no sea posible criar en grandes cantidades debido a la larga duración de su ciclo de reproducción.

0 INTRODUCTION

The literature of genetics and cytology dealing with the effects of radiation on insects have been reviewed by MULLER (1940, 1941) [16,17], LEA (1947) [14] and CATCHESIDE (1948) [4]. Valuable reviews of the literature on the action of ionizing radiation on insects, each including more than 200 references, have been given by HILCHEY (1957) [6] and GROSCH (1962) [5]. According to the findings reported, X-rays and gamma-rays cause similar effects. The extent of the changes in the germ cell depends on the dosage. Extreme doses cause the cell to degenerate. Less extensive changes may not prevent the sperm from fertilizing an egg, but the zygote usually dies in the embryonic stage. Mutations that prevent the survival of the fertilized egg are dominant lethal mutations. While the males irradiated at dosages sufficient to induce dominant lethal mutations in all the germ cells are for practical purposes sterile, since their progeny dies as embryos, they are not, technically speaking, truly sterile, because they are still capable of producing sperm which fertilizes eggs.

Of greatest economic importance is the extensive work done on the screw-worm, Callitroga hominivorax, by BUSHLAND et al. (1951, 1953) [2,3], BAUMHOVER (1955) [1], KNIPLING (1955) [12], and LINDQUIST (1955) [15]. Most of the extensive irradiation research on Coleoptera concerns pests of stored products. Only a few pests of field crops, e.g. bollworms, weevils, cane and corn borers, have been considered so far [13].

01 Biology of the cockchafer (Melolontha vulgaris F.)

In the regions concerned, most individuals of a natural population require three years for their development from egg to adult. The flight period of the adult cockchafer is concentrated in a few weeks every three years. During their flight, which usually starts about 20 April and continues through May and June, the beetles gather along the edges of or inside woods. Trees with deciduous foliage, such as oak and beech, are preferred host plants. After feeding for a short preoviposition period and mating, the females return to the adjacent fields. Oviposition occurs preferably in grassland. The chief crop losses have to be expected in the first and sometimes in the second year after the flight period. White grub damage mainly affects root vegetables, such as potatoes and sugar beet, strawberries and nurseries. Metamorphosis is completed in the summer of the second year after flight. The beetles hibernate in the soil near their exuviae at a depth of 20 - 30 cm. In the spring of the third year after the flight period, the three-year cycle

starts again with oviposition. The appearance of the first beetles may be predicted precisely by a calculation based on air temperature. The daily averages above + 8°C, beginning from 1 March, are added and when the sum of 256 ± 16°C is reached, the first mass flights can be expected (HORBER, 1955) [8]. A special behavioural feature favourable to the application of the sterile-male technique is the appearance of males in large numbers in advance of the females. A natural separation already takes place in the soil, most likely as the result of a differential threshold of sensitivity to soil temperature as between the two sexes (HORBER, 1955) [8].
females, sometimes in proportions up to 40 : 1, in catches in light traps operated during the first hour after sunset (Table I). This artificial procedure superimposed on the natural separation yields a predominantly male fraction that consequently may be sterilized and released before the appearance of the females.

On the other hand, it has to be taken into account that each female is likely to mate several times. It has not, however, been proved that several matings are necessary or of biological significance in increasing reproduction.

TABLE I

SEX RATIO OF COCKCHAFERS OBSERVED IN A LIGHT TRAP

1959 VENDLINCOURT				1962 'LES TAYES'			
Evening operated	Cockchafers trapped		Ratio M/F	Evening operated	Cockchafers trapped		Ratio M/F
	Males	Females			Males	Females	
6 May	10	35	0.3	3 May	71	26	2.7
7	80	45	1.8	7	116	35	3.3
8	124	31	4.0	12	143	142	1.0
9	44	1	44.0	16	719	210	3.7
10	43	7	6.1	21	294	205	1.4
13	2	0	∞	22	65	85	0.7
Total	303	119	2.5	Total	1408	703	2.0

02 Conventional methods of controlling cockchafer and white grub

Both chemical and mechanical means of controlling this pest, in either the larval or the adult stage, are available. Several cultural methods of preventing white grub damage are recommended (HORBER, 1954, 1958, 1961) [7,9,11]. In a number of Swiss cantons the collection of May beetles had been declared obligatory and has been carried out in some places for a century or even longer. Since 1948, several attempts have been made on a large scale to eradicate the cockchafers by treating the woods and also dispersed trees preferred as food with insecticides.Compounds based on lindane proved to be the most effective and were therefore widely used. Insecticidal treatments on a large scale during a short flight period require the avail-

ability of a considerable number of atomizers or, in difficult country, of aircraft. Very close co-operation among a well-trained technical and biological staff is necessary.

1 LABORATORY EXPERIMENTS ON THE NECESSARY X-RAY DOSAGE AND IRRADIATION EFFECTS

11 Material and methods

From 1955 to 1958 samples of cockchafers were taken in three different regions of Switzerland. They were obtained in March and April by digging them out of their hibernation sites. They were kept at temperatures below + 6°C. No food was provided before treatment. The irradiation was performed by the staff of the radiobiological laboratory of the Zürich Cantonal Hospital. The X-ray apparatus used was a Siemens "Stabilivolt" operated at 180 kV and 6 mA. During the exposure the beetles were confined to a plastic phantom in lots of about 30 to 60. Before and after treatment they were kept in lots of about 20 in small tin or aluminium cans refrigerated at 4-6°C. The boxes were filled with wet sawdust. They served equally well as transport containers.

In order to measure fertility the irradiated males were exposed in cages along with an aliquot of unsterilized females. The fecundity of females after mating was measured by counting the eggs laid into the soil layer exposed on the bottom of the cages. Fertility was recorded by exposing equal batches of 20 - 25 eggs in wet soil or vermiculite in Petri dishes at 20°C. The young white grubs were counted and removed at regular intervals.

12 Results

The X-ray dosages administered are tabulated against fecundity and fertility (Table II). It was established from these counts by interpolation that 3000 r would be the minimum dose required to obtain at least temporary sterilization. Since mortality and behaviour during copulation were not appreciably affected up to a dosage of 20 000 r, it was assumed that it would be safe to administer a dosage within the range 3000 - 5000 r.

2 APPLICATION OF THE STERILE MALE TECHNIQUE TO A NATURAL POPULATION

21 Material and methods

211 Selection of the sites serving as treated, control and "bank" areas

An opportunity to apply the sterile-male technique to a natural population of the cockchafer was recognized in the Ajoie (Canton of Berne) where gradation had been observed carefully since 1953 in an area measuring about 2 500 ha. In 1950, an insecticidal treatment had been performed in the same district in order to control an outbreak of this pest. In the subsequent flight periods a gradual regression has been observed (Table III, Figs. 1 and 2).

Five different areas were selected, each containing about 30 ha of arable land, with 13 - 15 ha of grassland and other field crops and adjacent to woods. Each area was selected to be at least 3 - 5 km from the next in order to minimize the possibility of migrations. The main criterion for

TABLE II

EFFECT OF IRRADIATION ON REPRODUCTION OF THE COMMON COCKCHAFER

Year	X-ray dose applied (r)	Number of irradiated males	Number of eggs laid by an aliquot number of non-irradiated females	Eggs hatched (%)
1955	0	27	83	56
	5 000	27	66	0
	10 000	27	84	0
	20 000	27	84	0
1956	0	30	280	10.7
	1 000	29	160	1.9
	2 000	28	126	0
	4 000	29	95	0
	8 000	29	76	0
1957	0	64	307	45
	2 000	60	637	8.7
1958	0	65	205	56.6
	2 000	63	263	5.3
	4 000	62	94	0

TABLE III

GRADATION OF COCKCHAFER AND WHITE GRUB POPULATION IN THE AJOIE FROM 1950 TO 1962
(Average number per m² in the whole region)

Year	1950	1953	1956	1959	1962
Cockchafers	-	4.9	2.05	0.69	0.91
White Grubs	59.6*	15.8	13.96	1.50	0.48

* SCHENKER [18]

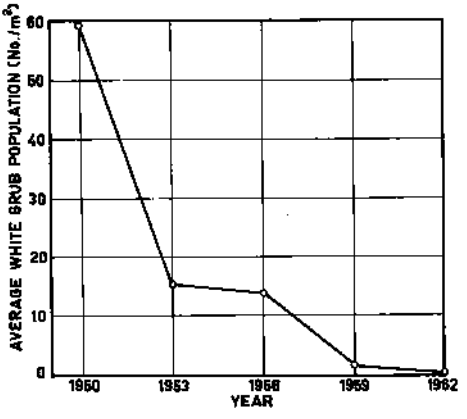

Fig. 1

Gradation of white grub population 1950 to 1962

Average number of white grubs per m^2 based on 350 sampling units in 1950, 496 in 1953, 1120 in 1956, 800 in 1959 and 864 in 1962.

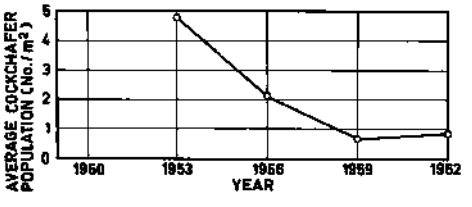

Fig. 2

Gradation of cockchafer population 1953 to 1962

Average number of cockchafers per m^2 based on 928 sampling units in 1953, 864 in 1956, 800 in 1959 and 688 in 1962.

selecting these areas for the field trial was a moderate population density both in the area to be treated and in the untreated or control areas, with a higher density for the area selected as the "bank". In the description below these areas carry the following designations (see Fig. 3):

Area A, the treated area, where males were captured, irradiated and released (Vendlincourt);

Area B, the "bank", where cockchafers were collected and the males irradiated for release in area A (Les Tayes);

Area C, the control area, where undisturbed gradation was observed (Alle = C_1, Coeuve = C_2, Lugnez = C_3).

212 Description of the areas selected for the field experiments 1959-62

The rural district surveyed since summer 1950 is situated on the north-western foothills of the Bernese Jura ranges. The terrain stretches over a rolling landscape underlaid by Jurassic limestone and is irregularly dotted

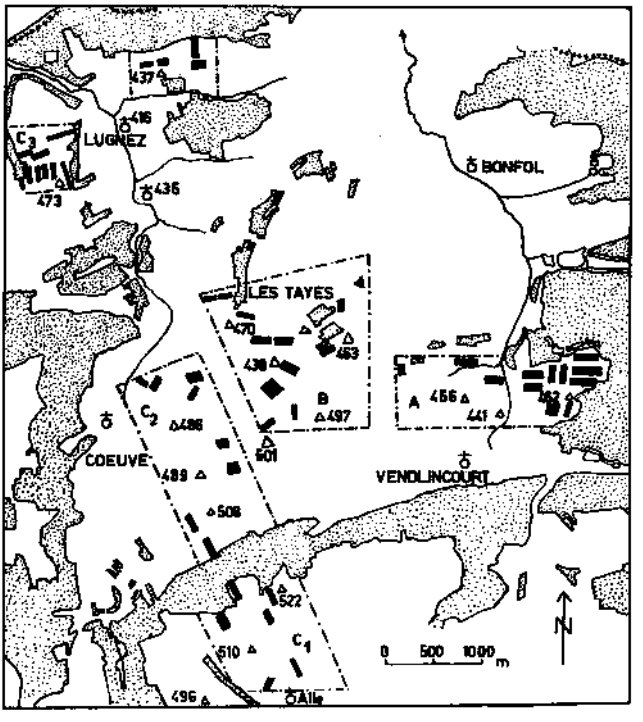

Fig.3

Field Experiment 1858 to 1962

Boundary lines -.-.- of areas A (sterilized males released, B(bank), C1-3(controls), with positions of grassland plots indicated, in each of which 16 sampling units of 0.25 m² were dug in order to estimate white grub and cockchafer populations. Small numerals indicate altitude (m above sea level).

with dolines, marked with oaks, beeches and cherry-trees. The altitude ranges from 420 to 520 m above sea-level. The villages are situated at the bottom of the valleys. The tops of the hills and some of the steeper slopes are wooded. The whole region is surrounded by woodland. Intensive farming is confined to the bottoms of the valleys, while in the higher grassland and pasture areas the arable land is predominantly planted to wheat, oats and barley. The soil consists of renzina, loam and sandy loam. Horse-breeding has been a traditional activity in this district.

The treated area A is situated near Vendlincourt. On three sides it is surrounded by woodland while to the west it is separated from the neighbouring farmland by a belt of poorly drained grassland. The enclosed area, measuring about 30 ha of open land with 14 ha of grassland, is marked out from the other areas by its lighter sandy-loam soil. During our first survey in 1953, we noticed that the mortality of the cockchafers between oviposition and flight was lowest in that area (Table IV). Farmers in that region

TABLE IV

MORTALITY CALCULATED BY COMPARING COCKCHAFER POPULATION WITH WHITE GRUB POPULATION AFTER THE PREVIOUS FLIGHT PERIOD

Area	Period of observation		
	1953 - 56	1956 - 1959	1959 - 62
A	78.9	87.6	68.8
B	84.1	91.2	63.1
C	89.7	95.8	43.8

observed that this soil was also a preferred hibernation site for the Colorado potato beetle (Leptinotarsa decemlineata Say). We therefore surmised that this area might possibly serve as an infestation centre from where outbreaks of the cockchafer may spread over the surrounding areas.

The adults emerging from the grassland in this area collected along the border of a triangular wood. One corner of this border is raised on the rim of a hill and protrudes towards the open area; it is therefore highly attractive to the emerging beetles. The wood consists of oak, beech, cherry-trees and poplar, which are preferred food trees.

The "bank" area B has a unique topographical situation in that it consists of a group of small woods called "Les Tayes". Situated on top of a hill, they attract the cockchafers from all directions over a great distance. Although the soil of the surrounding fields consists of renzina, which is not good breeding ground for white grub, cockchafers emerging from several 100 ha of the adjacent slopes are attracted to these woods. As these contain preferred food trees, they act as bait and consequently great masses of beetles may be collected along their borders.

The control areas C: Three areas situated near Alle, Coeuve and Lugnez respectively were selected because of the similarity of their topographical situation and the renzina soil they have in common. The fields and grassland from which the beetles emerge are situated on slopes topped with woodland. Near Alle the slope is exposed to the south, near Coeuve it is situated to the west, while near Lugnez a small wood stretched out along the top of a ridge attracts beetles from both hillsides. In many fields of these areas the underlying limestone of the Jura formation is barely covered by a thin renzina layer and the are therefore exposed to drought conditions sooner than the areas A and B.

Sectors of about 30 ha of cultivated surface together with the adjacent wood in each of these three districts were considered as control areas, where the undisturbed development of the population was observed from 1953 to 1962.

213 Estimation of the cockchafer and white grub populations

Several weeks before the flight started the population density was estimated by digging and searching through about 800 sampling units each consisting of 0.25 m^2 of surface area down to a depth of 20 - 40 cm. Between 112 and 256 such sampling units were dug in each of the five areas, depending on their size. The samples were grouped and classified according to the distribution of grassland in the area. In each area 7 - 16 plots of grassland or fields of about 0.4 - 0.6 ha surface were sampled. In each plot 16 random 0.25-m^2 sampling units were averaged. The number and sex of beetles were recorded separately for each plot.

In 1959 the procedure was repeated in order to estimate the white grub population during late summer and autumn of the same year. The sampling units were dug in the same plots and fields as in the previous surveys. The plots were numbered for identification purposes and their size was marked on a sketch-plan for each area and also on 1 : 25 000-scale survey maps for the whole region of operations. The figures in Tables III and IV have been compiled from a total of 6900 sampling units.

These counts served for the calculation of

(a) The population density of cockchafers just before the flight period, their sex ratio and their distribution over the area.
(b) The population density of white grubs after the flight period and their distribution over the area.
(c) The rate of reproduction after the flight period, obtained by dividing the number of white grubs counted in a given plot or area by the number of cockchafers counted in the same plot or area before the flight period.
(d) The mortality in the three years between successive white grub or cockchafer counts.

214 Labelling procedures

The cockchafers collected in the area A or B by shaking their host trees or by operating light traps were confined in wire cages and transported to the field laboratory. There they were sexed and the males were marked with a leather dye. This consisted of an adhesive pure-white base with which other colours could be mixed. The beetles were labelled individually by applying the dye with small brushes as spots on the thorax or on the last abdominal tergum. When sufficiently dry, the beetles were packed into cardboard boxes in lots of about 200. These boxes were filled with wet vermiculate and then refrigerated to +4 - 6°C.

In 1962 the females captured in areas A and B were labelled with P^{32}. The following large-scale procedure was adopted. A solution of 8.9 ml NaH_2PO_4 having an activity of about 40 mc at 4 p.m. on 2 May was diluted with 5 l water and mixed with 10 l vermiculite. This mixture, containing 2.65 μc/ml, was poured in a plastic container with a cover. The female cockchafers were added to this labelling mixture in lots of several hundreds at a time. The beetles stayed in the mixture for a day, during which time the container was thoroughly shaken and rolled at intervals in order to secure complete contact between the beetles and the radioactive mass. When measured with a portable α - β - γ survey-meter (Tracerlab with a TGC-6 Probe) the freshly labelled beetles showed activities ranging from 200 to over 2000 counts/min. A count of 100 counts/min in an indi-

vidual was regarded as sufficient to distinguish between labelled and unlabelled beetles during screening procedures under unshielded conditions in the field, with a background of 30 - 50 counts/min. These P^{32}-labelled females were released only in area B.

215 Irradiation procedures

The beetles were stored and transported as indicated in sub-section 214 above to the nearest X-ray unit, which was at the Bürgerspital, Basle, 80 km from the field laboratory. The beetles were exposed in lots of 400 - 600 at a time. They remained in the same boxes of 13×18 cm surface by 5 cm depth for the whole trip, including exposure, until they were released in the fields. The X-ray unit used was a Siemens "Stabilivolt" operated at 200 kV and 20 mA. The focus distance and irradiation time were set for a dose-rate of 665 r/min or a dosage of 3325 (Fig.4) (Table V). Simultaneous dosage measurements were supervised by the radiophysicist of the Institute of X-ray therapy at the University of Basle. Each trip to and from the X-ray unit, including irradiation, took about four or five hours.

TABLE V

ESTIMATE OF THE COCKCHAFER POPULATION BASED ON SAMPLING IN GRASSLAND BEFORE THE FLIGHT PERIOD 1959 IN FIVE AREAS OF THE AJOIE FIELD EXPERIMENT

Area	Number of		Average number of cockchafers per m^2	Maximum quantity of males expected(1)
	plots	sampling units		
A	16	256	0.48	62
B	10	160	1.15	> 1000
C_1	6	96	0.12	16
C_2	7	112	0.54	65
C_3	7	112	1.00	130

216 Release of the irradiated cockchafers

The irradiated and marked males were usually released during the evening of the same day in area A. As the maximum flight activity of the natural population started twenty minutes after sunset, the irradiated beetles were released from the refrigerated cardboard boxes and poured into large wooden cases filled with a handdeep layer of coarse, moistened sawdust so as to be ready at sunset. In some exceptional cases we had to postpone the release to the next morning as the result of delays in handling or bad weather during the evening.

The releasing cases were exposed in grassland at about 250 m from the nearest border of wood described in sub-section 212. These cases were camouflaged with oak or beech twigs. As an example of such a release,

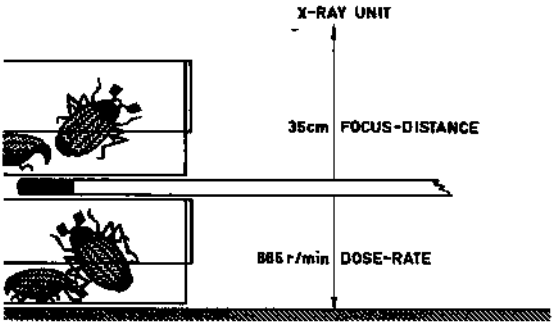

Fig. 4

Irradiation of male cockchafer for sterilization

Position of dosimeter during dosage measurements between two cardboard boxes each containing 200 males embedded in moistened vermiculite and kept below +6°C.

the one carried out on 12 May 1959 may be described. At 11.30 a.m. a total of 1504 males were exposed for release. Of these, about 850 left the cases during the first half hour. About 180 of them could be counted arriving at a prominent corner of the wood. Sunshine and a light northerly wind prevailed at this time; the temperature reading 2 m above the grass was 22°C.

At regular intervals the cases were checked for dead and remaining live males. The mortality was recorded. According to the counts taken at three different dates during the 1959 operations 89 - 94% of the beetles had left the releasing place on their own wings. The corresponding figures for the releases in 1962 were 74 - 98%. Bats and birds were consuming considerable numbers of cockchafers.

22 Results

221 Recapture of labelled males

Of 3109 males released in 1959 in area A, 202 individuals or 6.5% were recovered in light traps or collections along the woods. The corresponding figures for 1962 for the same area were: 8594 males released, of which 90 or 1% were recovered. Of the 2571 cockchafers labelled with dye and released in 1959 in area B, 22 or 0.9% were recaptured. Of the 6231 females labelled with P^{32} in 1962 in area B, 288 or 4.6% were recaptured. In 1959 the beetles in the two adjacent areas A and B had been labelled with different colours. In 1962 the males released in A were labelled with dye, whereas the females released in B were labelled with P^{32}.

No migration was observed in the two years either between these two areas A and B, or between those and any of the areas C.

222 Ratio of sterilized to normal cockchafers

Males were recaptured in the area in order to calculate the ratio of sterilized to normal cockchafers. During the period 10 - 14 May 1959 3109 irradiated males were released. In a sample of 514 beetles collected,

192 or 37.4% were labelled. For the last period recorded from 12 to 14 May 1959 the corresponding ratio was 52%. In 1962, for the first period recorded from 8 to 12 May, this ratio was 15 labelled to 18 recaptured or 83.3%. For the following period from 13 to 17 May 1962, the ratio was 30 to 30 or 100%.

223 Estimate of the cockchafer populations by the isotopic dilution technique

Cockchafers were recaptured in areas A and B in order to verify the estimates of the population as obtained by the sampling before flight had started. The formula used to calculate the population feeding and mating on the borders of the woods by the isotopic dilution technique was:

$$Q = q\,[(nl/r) - 1]$$

where Q = total number of individuals of the population;
q = total number of released and labelled individuals;
n = percentage of normally behaving individuals among total number released;
l = percentage of labelled individuals among total number released; and
r = percentage of labelled individuals in samples taken after release and intermingling.

The number of beetles released had to be corrected for those which lost the label, died or remained in the cases. The correction factor l was determined by the persistence of the label on the cockchafer. The persistence of the label had been checked at regular intervals on the cockchafers remaining in or near the releasing cages. The percentage of cockchafers which lost their labels could be reduced from 36% at the start of our operations to 0.2% at the end of them, with an average of 6%. The vitality of the released cockchafers is discussed in sub-section 215.

The estimate of cockchafer population may be compared in Table VII for the areas A and B in 1959 and in Table VIII for the same areas in 1962.

224 Effect of the first release of sterile males in 1959 on the subsequent white grub population

A remarkable reduction in the white grub population was obvious after the first release in A (see Table IX). The average number of larvae was 0.29/m^2. All plots had infestations far below 20/m^2, which may be regarded as a reliable indication of no damage in grassland. The white grub population in A dropped to 1/5 of that in the control areas and to 1/16 of that in area B.

The rate of reproduction calculated was less than unity in A, whereas in B and C the population had increased by factors of 2.6 and 2.7 respectively.

225 Effect of the second release of sterile males in 1962 on the subsequent white grub population

In 1962 not a single white grub was found in A. The number of cockchafers had increased in the other areas (see Table VI) as a result of an unusually low mortality rate in the period 1959 - 1962 (see Table IV), but

TABLE VI

ESTIMATE OF THE COCKCHAFER POPULATION BASED ON SAMPLING IN GRASSLAND BEFORE THE FLIGHT PERIOD 1962 IN FIVE AREAS OF THE AJOIE FIELD EXPERIMENT

Area	Number of plots	Number of sampling units	Average number of cockchafers per m^2	Maximum quantity of males expected (1)
A	12	196	0.1	13
B	10	160	1.3	> 1000
C_1	3	48	0.4	52
C_2	8	128	1.2	156
C_3	10	160	1.4	182

TABLE VII

ESTIMATE OF THE COCKCHAFER POPULATION IN 1959 IN THE AREAS A AND B DURING FLIGHT BY THE ISOTOPIC DILUTION TECHNIQUE BEFORE THE FIRST RELEASE OF STERILE MALES

Area	Period of observation	Sex	Individuals released	Labelled individuals recaptured	Correction[a] factors n (%)	Correction[a] factors l (%)	Proportion of labelled individuals (%)	Estimated population (No.)	Estimated population (l)
A	6-14 May	♂	3109	130	92	94	17.1	12620	25
		♀	1619	72	-	-	11.1	10934	22
Totals			4728	202	-	-	14.2	23744	47
B	10-14 May	♂	0	0	-	-	0	73258[b]	147
		♀	2571	22	97	94	5.7	38557	77
Totals			2571	22	-	-	1.96	116722	233

(a) Correction factor : n = % normally behaving individuals among total released.

l = % labelled individuals among total released.

(b) Estimate based on sex ratio of recaptured individuals ♂: ♀ = 1.9.

flight and oviposition were severely restricted by the unusually cold spring of of 1962. Egg development and hatching were prevented by drought. The reproduction rate in the whole region dropped below unity. The differences in infestation as between A and the other areas therefore appear less remarkable than they might have done.

TABLE VIII

ESTIMATE OF THE COCKCHAFER POPULATION IN 1962 IN THE AREAS A AND B DURING FLIGHT BY THE ISOTOPIC DILUTION TECHNIQUE BEFORE THE SECOND RELEASE OF STERILE MALES

Area	Period of observation	Sex	Individuals released	Labelled Individuals recaptured	Correction[a] factors n (%)	Correction[a] factors l (%)	Proportion of labelled individuals (%)	Estimated population (No.) (l)	
A	4-16 May	♂	8594	90	97.8	93.5	76.3	1 699	3.5
		♀	0	0	-	-	0	2 005	4
Totals			8594	90	-	-	63.8	3 704	7.6
B	9-28 May	♂	0	0	-	-	0	24 271	48
		♀	6231	288	98.3	91.2	16.4	27 814	56
Totals			6231	288	-	-	9.58	52 085	104

(a) Correction factor : n = % normally behaving individuals among total released.

l = % labelled individuals among total released.

(b) Estimate based on sex ratio of recaptured individuals ♂ : ♀ = 1.9.

3 DISCUSSION

31 Prospects for successful application of the sterile-male technique to the elimination of white grub

Although it is possible to rear white grub and cockchafers in the laboratory, with the aid of a recent technique, in a shorter time than in nature (HORBER, 1959 [10]) it will hardly be possible to produce them in quantities large enough to outnumber the males of a natural population.

The basic strategy would be to capture males in areas where they are readily obtainable in large numbers and, after sterilizing them, to release them in the areas to be treated.

Thus the release of radiation-sterilized males enters the picture where a population is at a moderate-to-low level, either as a consequence of a regression in the course of natural gradation or following a more or less successful application of the other control measures. Even if not feasible for use alone, the method might be of importance in combination with others. Sterilized males might, for instance, be released to apply the coup de grâce to a population that has already been depleted by other control measures and in which the survivors could not be further reduced by any other practical means ; or this technique might be usefully applied to a population in the prodromal stages of an outbreak.

It is believed that this technique would not upset the natural balance or do direct harm to beneficial insects, bees, birds or fish, as has frequently

TABLE IX

EFFECT OF THE FIRST RELEASE OF STERILE MALES IN 1959 ON THE SUBSEQUENT WHITE GRUB POPULATION.

Estimate of the white grub population and of the rate of reproduction

Area	Number of plots	Number of sampling units	Average number of white grubs per m^2		Rate of reproduction	
A	14	224	2.29		0.52	
B	15	240	5.51		2.61	
C_1	7	112	1.29		12.00	
C_2	8	128	2.87	1.55	6.29	2.73
C_3	13	208	0.87		0.74	

TABLE X

EFFECT OF THE SECOND RELEASE OF STERILE MALES IN 1962 ON THE SUBSEQUENT WHITE GRUB POPULATION

Estimate of the white grub population and of the rate of reproduction

Area	Number of plots	Number of sampling units	Average number of white grubs per m^2		Rate of reproduction	
A	15	240	0		0	
B	11	176	0.91		0.77	
C_1	8	128	0.41		2.60	
C_2	10	160	0.78	0.57	0.82	0.65
C_3	10	160	0.50		0.36	

been observed after chemical treatments. No insecticide residues in grass and milk, nor off-flavour in potatoes or in other crops, need be expected, in contrast to experience with chemical treatments. This technique might also be useful in areas where cockchafers or white grubs have developed a high degree of resistance to insecticides.

32 Future problems

In the field experiment reported, the irradiation unit was situated far away from the release point of sterilized males. The irradiation time was therefore limited by the transport facilities and by the short time the unit was available for work with cockchafers, usually only at the end of a tight

schedule of the daily routine of X-ray therapy. The desirability of obtaining a mobile irradiation unit, with no other obligations while on duty for sterilizing work, is emphasized. The unit should be available in the immediate neighbourhood of the points of release. It would allow larger masses of insects to be treated in a much shorter time. This would enable larger areas to be covered or alternatively to be treated at higher infestation levels.

Furthermore, the basic strategy has to be considered and differentiated in a higher degree according to the ecological conditions or the farming system encountered in the area involved.

In our future work with cockchafer we plan to differentiate between areas with highly susceptible crops from other which tolerate a moderate degree of infestation. Areas where plants of zero tolerance are grown, e. g. potatoes, sugar beet, strawberries, vegetables, vineyards, orchards and nurseries, must be very carefully protected from white grub damage. Such areas have to be protected by an immediate reduction in the white grub population in the course of a single season. This involves manipulating the irradiation dosages so as to ensure a safe permanency of sterility in released males. In areas with grassland, cereals or other crops, which in contrast tolerate a higher infestation level, or where the cultures suffer from white grub damage only in the following season, the permanency of male sterility might be neglected in favour of the possibility of spreading a fair amount of sub-lethal genes among the surviving white grub population.

With regard to cockchafer and white grub, a field of application of the sterile-male technique appears to be in Alpine valleys, where the requirement of geographical isolation is usually fulfilled to some degree, where the terrain is difficult for the conventional control methods, and where the cultivated area is mostly limited to the bottom of the valley and is too small to support the high costs involved in treating the extended forests on both slopes. Sterile males released at well-chosen points would presumably operate better, by finding females and destroying their fertility in barren areas, than any surface-bound or airborne carrier of insecticide.

However, many more details of the sterile-male technique as applied to cockchafer, or any other insect pest, remain to be investigated more carefully. Thus it seems reasonable for leading workers to give such projects high priority in their schedule of activities, since this appears to be not merely another ingenious device of biological and technical progress in pest control, but in many respects a potential breakthrough.

33 Epidemiological studies facilitated by the sterile-male technique

It will be interesting to study the manner and speed with which areas such as area A, where white grub has disappeared after repeated releases of sterile males, become reinfested. Does reinfestation depend on immigration or on the multiplication of a few survivors? Another interesting task would be to determine the minimum size for viability in an area of a cockchafer population.

4 SUMMARY

Laboratory tests indicated an X-ray dose of 3000 r to be sufficient to induce sterility in male cockchafers. During two flight periods, sterilized males were released among a natural population in order to eliminate white grub in a mixed farming region of north-western Switzerland. In 1950 an outbreak of this pest was reduced by a chemical treatment. Gradation has been watched during every flight from 1953 to 1962.

In 1959 five areas, each with a surface of about 30 ha, were selected to serve as:

A treated area A, where males were captured, irradiated and released;
The "bank", B, where cockchafers were collected and the males were irradiated and released in A;
Three control areas, where undisturbed gradation was observed.

The males were irradiated in a therapeutical X-ray unit. Irradiated males were handpainted so that the ratio of sterilized males could be estimated by the isotopic dolution technique.

In 1959, for the first treatment, about 6 l of sterilized males were released in A, representing about 50% of the male population. The white grub infestation sampled in grassland dropped thereafter to about 1/5 of that in the other areas. Reproduction rate was less than unity only in A. A further reduction of the population in A to 1/10 of that in B and C was observed when the number of surviving cockchafers was estimated in 1962. The greatest mortality from 1959 to 1962 occurred in A.

In 1962, for the second treatment, a total of 17 l of irradiated males was released in A. At least 76% of the male population of A was sterilized. Subsequent sampling of the white grub population showed complete eradication in A. Some reduction was also observed in B and C as a result of drought in the whole region.

It has been demonstrated that the sterile-male technique may successfully be applied to an insect pest in an area which is not strictly isolated geographically, the females of which mate several times and the artificial breeding of which in large quantities is not feasible because of the long breeding cycle.

ACKNOWLEDGEMENTS

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The author is much indebted to Professor H. Fritz-Niggli and to Miss Berger for valuable advice and assistance during the irradiation experiments at the Radiobiological Institute of the Zürich Cantonal Hospital and to Professor E. Zdansky and to Dr. H. Lüthy for giving permission to use the facilities, to survey the irradiation procedures and to perform dosage measurements at the Bürgerspital, Basle.

The extended fieldwork involved was vigorously promoted by the agronomy engineers Messrs. J. Huber, R. Schoch, E. Wüst and A. Fekti. Mr. W. Schoch and Mr. P. Keller participated in the practical field work. Dr. Bögli arranged for the help of some students of the Hitzkirch seminary. I take this opportunity of thanking them for their careful work and endeavour.

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DISCUSSION

M. FERON: I would like to mention that the cockchafer is a particularly favourable insect for this type of experiment. Professor Stone indicated earlier that a strict isolation of the areas concerned is needed in order to obtain success*. In the case of the cockchafer, the female comes out of the fields to go to the trees for mating, and afterwards it goes back exactly the same way in order to oviposit. The movements of the females are thus restricted and this insures a certain behavioural isolation. The males, of course, do not behave in the same way, since they do not lay eggs. I think this contributes considerably to the success of such an experiment.

E. HORBER: That is quite correct. The behavioural studies of my colleagues Couturier, Roberts and Schneider show that at moderate population levels behavioural isolation can be obtained even without geographical isolation.

W. KLOFT: In regard to the sex ratio of cockchafers, the figures in Table I show more males than females. Several times in Würzburg we collected Melolontha vulgaris and we always found an exact 1:1 sex ratio. But in your case the insects were caught with light traps. Perhaps that explains the different results obtained.

E. HORBER: Yes, these figures given in my report apply only to insects caught in light traps, during the flight period; moreover, they are possibly peculiar to the locality where the light trap was operated. The sex ratio of a natural, undisturbed population can best be checked by digging

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* See discussion of STONE, W. E., Effects of ionizing radiation on insects and other arthropods, these Proceedings.

the beetles out just after metamorphosis during the autumn, before the flight period. The ratio may then be expected to be exactly 1:1. In our survey work during many years at different localities it was always exactly or very nearly 1:1 as verified by the χ^2 test. During the flight period, however, a continuous dynamic process is going on whereby the sex ratio may change very rapidly depending on several factors, e.g. phase of oviposition, higher mortality rate of the males, etc.

G.B. VIADO (Chairman): In the Philippines we have several species of white grub which are very destructive to crops. Extensive surveys were carried out many years ago because of the damage they do to sugar cane. We collected sacks full of these adult beetles by shaking them from trees and shrubs and we found a sex ratio of about 2 : 1 in favour of males.

A TECHNIQUE OF CULTURING THE OLIVE FLY, DACUS OLEAE GMEL., ON SYNTHETIC MEDIA UNDER XENIC CONDITIONS

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Abstract — Résumé — Аннотация — Resumen

A TECHNIQUE OF CULTURING THE OLIVE FLY, DACUS OLEAE GMEL., ON SYNTHETIC MEDIA UNDER XENIC CONDITIONS. Five generations of Dacus oleae have been cultured on an agar-dehydrated carrot base medium that contains an enzymatic protein hydrolysate of soya or casein, brewer's yeast, choline chloride and olive oil. Although the culture technique is xenic, an attempt is made to control microorganisms by chemical means. A bacterial species replaced the normal symbiote within D. oleae and was believed to be essential to maintaining the stock, but two generations of D. oleae have been cultured without any bacteria being present in the sites normally occupied within the larva or adult by its typical symbiote. Streptomycin is now being incorporated into the adult food to control bacterial infection of the eggs. Normal larval development, size and reproduction of D. oleae is obtained.

Mass culture is possible using the larval medium developed, but further research is necessary to find a faster method of placing the eggs on the medium. Further screening of mould inhibitors is desirable, as well as seeking cheaper substitutes for the medium.

MÉTHODE D'ÉLEVAGE DE LA MOUCHE DE L'OLIVE (DACUS OLEAE GMEL.) EN XÉNIE SUR MILIEUX SYNTHÉTIQUES. On a élevé cinq générations de Dacus oleae sur un milieu à base de gélose et de carotte déshydratée contenant un hydrolysat enzimatique de protéines de soja ou de caséine, de levure de bière, de chlorure de choline et d'huile d'olive. Bien que l'élevage se fasse en xénie, on s'efforce d'enrayer le développement des microorganismes à l'aide de moyens chimiques. Une espèce bactérienne a remplacé le symbiote normal chez D. oleae et sa présence semblait indispensable dans le lot d'élevage, mais on a élevé deux générations de D. oleae sans qu'aucune bactérie soit présente aux emplacements normalement occupés chez la larve ou l'adulte par le symbiote caractéristique. Pour éviter la contamination des œufs par les bactéries, on incorpore maintenant de la streptomycine au régime alimentaire des adultes. On a obtenu un développement larvaire normal et des insectes de taille normale, qui se reproduisent normalement.

Le milieu larvaire qui a été mis au point permet l'élevage en masse, mais il faudrait procéder à de nouvelles recherches pour découvrir une méthode plus rapide pour placer les œufs sur ce milieu. Il faudrait perfectionner les inhibiteurs de moisissure et mettre au point d'autres milieux d'un prix de revient moins élevé.

МЕТОДИКА КСЕНИЧЕСКОЙ КУЛЬТИВАЦИИ МАСЛИНОЙ МУХИ DACUS OLEAE НА СИНТЕТИЧЕСКОЙ СРЕДЕ. Пять поколений D.oleae культивировали на среде агар-обезвоженная морковь, содержащей энзиматический гидролизат соевого или казеинового белка, пивные дрожи, хлорид холина и оливковое масло. Несмотря на ксеническую методику культивации, сделана попытка контролировать микроорганизмы химическими средствами. Бактериальные разновидности заменяли внутри D.oleae нормальный симбиот, что, как полагали, являлось важным для сохранения вида. Однако у двух поколений D.oleae не обнаружено каких-либо бактерий в участках, обычно занимаемых внутри личинки или взрослой особи типичным симбиотом. Стрептомицин в настоящее время включается в состав пищи взрослой особи для предупреждения бактериальной инфекции яиц. Личиночное развитие, размеры и размножение D.oleae были нормальными.

С помощью разработанной личиночной среды возможна массовая культивация, однако необходимы дальнейшие исследования для создания более быстрого метода размещения яиц на среде. Желателен дальнейший отбор ингибиторов плесени, а также отмокание более дешевых заменителей среды.

UNA TÉCNICA DE CRÍA DE LA MOSCA DEL OLIVO (DACUS OLEAE GMEL.) EN UN MEDIO SINTÉTICO EN CONDICIONES XÉNICAS. Se han criado cinco generaciones de Dacus oleae en un medio a base de agar

y de zanahoria deshidratada que contenía un hidrolizado enzimático de proteínas de soja o de caseína, levadura de cerveza," cloruro de colina y aceite de oliva. Aunque la técnica de cría es xénica, se ha tratado de controlar químicamente el desarrollo de los microorganismos. Una especie bacteriana ha reemplazado el simbiota normal del D. oleae y su presencia parecía indispensable, pero se han criado dos generaciones de D. oleae sin bacteria alguna en los lugares que el simbiota característico ocupa normalmente en la larva o en el adulto. Para evitar la infección bacteriana de los huevos se añade ahora estreptomicina al alimento de los adultos. El desarrollo de las larvas y el tamaño y la reproducción de los insectos son normales.

El medio preparado para las larvas permite la cría en masa, pero sería necesario efectuar nuevas investigaciones a fin de descubrir un método más rápido para colocar los huevos en ese medio. También convendría estudiar nuevos anticriptogámicos y encontrar productos que permitieran preparar un medio menos costoso.

INTRODUCTION

Trypetid pest species are likely candidates for control or eradication by releasing sterile (lethal) males [1-9]. This technique is possible if a species can be mass-cultured.

Five trypetid species can to date be mass-cultured on prepared media not containing their natural host tissue. These species are the Mexican fruit fly, Anastrepha ludens Loew [10], the Mediterranean fruit fly, Ceratitis capitata Wiedeman [11-15], the oriental fruit fly, Dacus dorsalis Hendel [11-13], the melon fly, Dacus cucurbitae Coq. [13], and the Queensland fruit fly, Dacus tryoni Frogg [16, 17].

Dacus oleae Gmelin is also an important pest species. It causes extensive economic losses to olive growers in the Mediterranean region. In Greece alone, it is estimated to cause losses of about US $15 million annually [18, 19].

The Greek authorities became interested in conducting an experiment using the irradiation-of-male method of control against the olive fly on an island. Greece is ideally suited for such an experiment because of its numerous isolated islands bearing olive trees. The Greek Atomic Energy Commission consulted the United States Atomic Energy Commission as to the feasibility of such a test and the latter Commission sent two entomologists to Athens to investigate the problem.

These visiting entomologists met with a special committee consisting of entomologists and biologists from the Greek A.E.C., the University of Athens, the Department of Plant Pathology of the Ministry of Agriculture, the College of Agriculture of Athens and the Benaki Plant Pathological Institute. The committee was not only concerned with the sterile-male technique of control but outlined a research programme, (which included ecological studies, a search for new adult attractants and intensifying work on other methods of control), since any type of control that would reduce the average density of the olive fly would make it less difficult to obtain favourable overflooding ratios of released lethal males.

It was clear from the discussions with the committee that the olive fly would be amenable to the sterile-male technique. The monophagous nature of D. oleae, which attacks only olives, and its low abundance during certain periods of the year are attributes that perhaps make it better suited for the sterile-male release method than the other more tropical species of trypetids that have wider host ranges and more generations. The olive fly is indeed polygamous and more so than the other trypetids under investigation at

present. However, it is thought now that polygamy is not a critical factor for the lethal-male release method [20, 21].

Preliminary studies with dosages of irradiation necessary to induce dominant lethality in the sperm have been determined for the puparia, and cage tests with competitive normal males have given favourable results [4]. Radiation studies with other stages and puparia of D. oleae have recently been made in Greece [22].

It was plain to the committee in Greece that the main problem to be solved before an island test could be made using sterile D. oleae males was first to develop a mass-culture technique. Simultaneously further research on irradiation dosages and a search for effective adult attractants should be made.

The "Olive Branch Enterprise", as the committee had named their programme, requested an expert on fruit-fly nutrition through the International Atomic Energy Agency. In November 1961, K. S. Hagen was obtained from the University of California at Berkeley, California, and work was begun on the culture of the fly at the College of Agriculture in Athens.

RESEARCH APPROACH

There are at least three approaches that can be made toward culturing D. oleae. They can be classified as axenic, monoxenic and xenic. These terms refer to the number of organisms associated with the species that is under investigation as to its nutritional requirements [23].

The axenic approach to culturing D. oleae larvae requires that no other species of organism be present either in the larva or in its substrate. MOORE [24, 25], using aseptic techniques, attempted to use this approach, and was able to obtain adults. The developmental period was slower for the larvae than normal, and the resulting adults were reproductively weak.

It is possible with the technique Moore used that a monoxenic condition existed, i.e. that besides D. oleae one other species was involved in the culture, a bacterial symbiote. An extra-cellular symbiote, Pseudomonas savastanoi Smith, is found in nearly all wild D. oleae and is transmitted from generation to generation [27-29]. Even though Moore surface-sterilized the D. oleae eggs, some bacteria could have already entered the egg at the time of deposition.

The olive-fly larva with its symbiote within an olive is approaching a monoxenic condition, for usually the olive appears to be quite sterile at the larva feeding site. The larva prefers to eat undisturbed tissue as it tunnels through the fruit.

Recent experiments, which will be reported elsewhere in detail by the senior author, conclusively showed that, when adults of D. oleae which have been reared from olives are fed streptomycin along with the regular laboratory adult diet, eggs deposited in olives hatched. However, nearly all the larvae died in the first-instar stage after tunnelling considerable distances in the olive. No larvae attained the third instar.

Eggs obtained from these same adults and placed on an artificial medium containing an enzymatic protein hydrolysate completed their development normally, and the resulting adults deposited viable eggs. Thus the role of Pseudomonas savastanoi as a symbiote is perhaps to hydrolyse the protein

in the olive tissue ingested by the larva, making available some essential amino-acid or acids, or possibly to synthesize an amino-acid or acids lacking in the olive.

The diet that MOORE [25] employed for the larva was similar to the aseptic diets used for some other fruit-fly species [26], except that it contained a higher level of protein and sitosterol was used in place of cholesterol. The fact that no hydrolysed protein was in the diet Moore used possibly indicates that the symbiote was present in the larvae.

The approach used in Greece was a xenic one, which infers that the number of associated organisms in the larva or in the substrate is unknown. Since it would be difficult to mass-culture a trypetid species under aseptic conditions, a xenic approach is the only practical one. However, it is imperative that harmful contaminants be controlled. The technique of using an acid pH and mould inhibitors to control micro-organisms developed for the culture of other trypetids [26] was therefore used for D. oleae.

The rather acid pH of the medium controls many bacteria, including the normal symbiote P. savastanoi, but permits at least one other bacterial species to replace it. These alien bacteria may become pathogenic in older females and reduce fecundity by reducing the lifetime. Streptomycin was therefore incorporated in the adult food in an attempt to prevent bacteria from becoming established in the special morphological sites evolved to house the typical symbiote. The cycle of P. savastanoi and its sites in D. oleae have been well known since the classic work of PETRI [27]. (For other pertinent papers see [28, 29]).

Though the culture approach described below is a xenic one, it is nearing an axenic condition at least insofar as D. oleae harbours bacteria in its unique symbiote morphological sites. The inclusion of hydrolysed protein in the larval medium apparently permits D. oleae to develop without its symbiote.

MATERIALS AND METHODS

The temperature was usually 25 ± 1°C and the relative humidity was 75 ± 20%. Later the relative humidity was held at about 70% with the aid of a humidifier and a humidistat.

Adult handling

The type of cage used for stock flies is one that has been used for years in United States fruit-fly laboratories. About 200 flies were held in the 30-cm-cube cages (Fig. 1). The cages were placed near east-facing windows and exposed to normal diurnal light.

The adult food containing an enzymatic protein hydrolysate of yeast with a carbohydrate, and at times with brewers' yeast, is quite similar to the diets used in the culture of other trypetid species [30-32], and was effective for D. oleae egg production [25, 33]. The adult diet now used as a matter of routine in Greece is shown in Table I.

The diet shown in Table I, without streptomycin, was used to obtain eggs for most of the tests made. Only in later tests was streptomycin used and this will be indicated where appropriate.

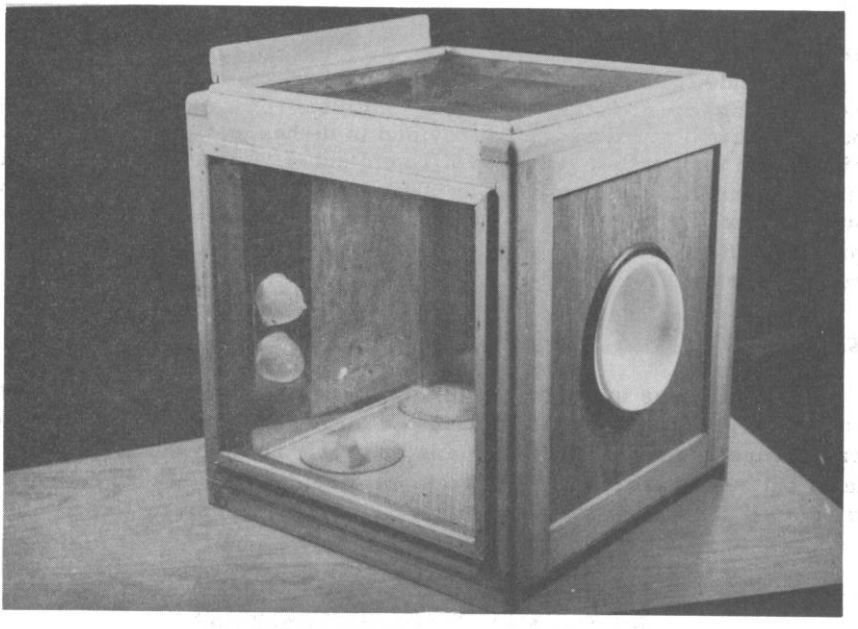

Fig. 1

Cage for adult Dacus oleae showing ovipositional sites (the paraffin-wax domes) and the feeding site (the wax paper) sealed inside on the front glass

TABLE I

COMPOSITION OF ADULT DIET FOR DACUS OLEAE

Substance	Amount (g)	Amount (ml)
Enzymatic protein hydrolysate of yeast*	20.0	
Sucrose	80.0	
Choline chloride*	0.1	
Streptomycin sulphate *	0.5	
Water		100.0

* Nutritional Biochemicals Corp., Cleveland, Ohio.

It is imperative that the adult food be supplied to the flies in a fluid form. Placing solid enzymatic protein hydrolysate yeast in the cage along with solid sucrose and water separately, the method used for feeding other fruit-fly species, is not effective with D. oleae. This species feeds very little on solid protein hydrolysates. It will feed, to some extent, on solid sucrose.

It appears that protarsal contact with the food is necessary to obtain a feeding response.

The liquid food is brushed in streaks onto wax paper which is sealed to the glass cage front facing the light. Solid sucrose and a water-soaked cotton-wool wad are separately provided in dishes on the floor of the cage (Fig. 1). In the smaller test cages (15 cm cube), where five pairs of flies were often used for determining fecundity, the food was provided in triplet form on wax paper on the floor of the cage, and once a day the food was sprayed with a fine mist of water. In the larger cages, it is necessary to brush on the food twice a day.

Egg handling

To obtain eggs in a suitable form for manipulation or counting, two domes of thin paraffin wax were used per large cage (Figs. 2 and 3). These domes were sealed to a slip of glass by gently heating the glass on a hot plate, but before sealing the glass a small wad of water-soaked cotton-wool was placed under each paraffin dome. This was necessary to prevent the

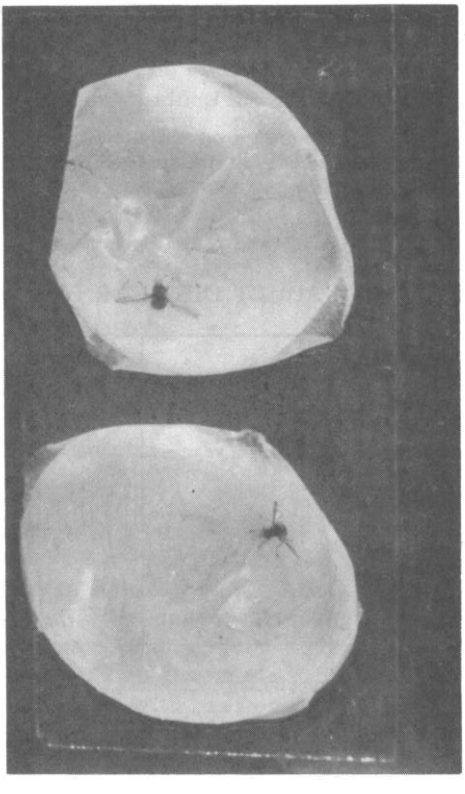

Fig. 2

Top view of paraffin-wax domes sealed to a glass slip showing Dacus oleae ovipositing

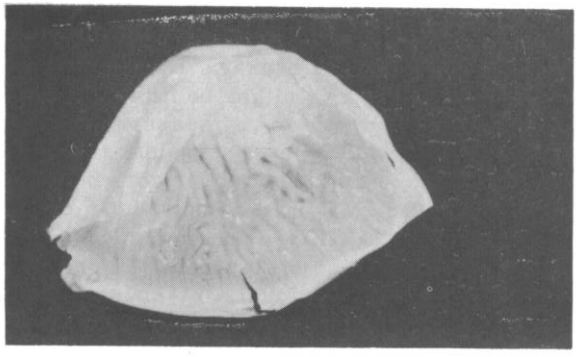

Fig. 3

Under-surface of paraffin-wax dome showing eggs of Dacus oleae at deposition sites

eggs from drying. The glass slip with the domes was fastened with cellulose adhesive tape to the front glass next to the food paper (Figs. 1 and 2). Fewer eggs were obtained if the domes were exposed on the floor of the larger cages.

The paraffin domes are easily made by using paraffin wax with a melting point of 52-53°C, or mixtures of paraffins with different melting points can be used as long as the resulting melting point is between 52 and 55°C. The best results have been obtained by adding 5-10% beeswax to the paraffin wax melting at 52-53°C. The paraffin is heated to between 70 and 90°C. A wooden mould of the size and shape of a hen's egg is first dipped into a thick soap solution and then plunged quickly first into the hot paraffin and then into water. The hardened paraffin shell is now easily pulled off the mould.

It is necessary to make new domes every day, for the used ones usually break when the eggs are removed from the under-surface (Fig. 3); also the domes become so perforated with ovipositional punctures that the eggs may become too dry.

Plastic moulds or thick wax forms, with or without ready-made punctures, have been used successfully with other trypetids being cultured but are not utilized by D. oleae for oviposition. This species does not oviposit in an existing hole, but drills, or attempts to drill, a new hole each time it deposits its single egg. Thus a very thin, easily penetrated material must be used in order to recover good eggs.

Often oviposition is attempted on any smooth, clean surface, like glass, and frequently an egg is deposited on this surface. These eggs often fall to the bottom of the cage and are lost because they soon become dry. Cages with screen bottoms permit the eggs to fall through into pans of water, and these eggs can be used. However, these eggs can easily become contaminated with microorganisms.

The paraffin domes are placed in the cages each morning and are removed in the afternoon or the next morning. The shorter the exposure to oviposition the better, since the range of hatching would be shorter and more uniform.

The eggs from the domes are collected in several different ways. The most rapid method is by dipping the domes into distilled water. The dislodged eggs fall to the bottom of the beaker. Excess water is decanted off, and the eggs, with some water, can either be filtered and caught by a fine nylon organdy, or , if the eggs are to be measured, they are drawn and pipetted into a funnel that has its tip pressed against a piece of nylon organdy which rests upon the surface of a large rubber cork held by a clamp. The eggs are measured volumetrically in the funnel tube and spread out over the surface of the organdy by brushing. The nylon pad with eggs is placed on moist filter paper and kept moist until hatching begins. For many experiments the eggs were removed individually by brush from the domes and placed on moist filter paper.

The incubation period is about 2.5 d at 25°C. The Petri dishes containing the eggs on wet filter paper must be clean and kept as free as possible from invasion by microorganisms.

A copper chloride solution of 1:1000 was used for a time in the egg dishes to control moulds and yeasts, but this was discontinued since the adults resulting from the eggs often died prematurely.

At present some mould inhibitors are being tested in the egg-holding dishes in an attempt to control yeasts and moulds that arise from egg placement on the medium.

Eggs exhibiting larvae with visible mouth hooks, and larvae which have just hatched, are placed on the media. Larvae one day old become too weak to be used. In earlier tests the egg pad was placed upon the smooth surface of the medium, and the larvae would enter the medium through the cloth mesh. However, eggs in contact with each other often would not hatch. The oil from the medium seeping up through the pad may have accounted for this egg mortality.

A higher percentage of hatch and puparial recovery is obtained when the egg or larva is placed individually directly upon the medium by brush. There is less chance of the medium becoming heavily contaminated than when an egg pad is placed on the medium. The water carried by the nylon egg pad evidently dilutes the mould inhibitors and alters the pH, allowing growth of unwanted microorganisms.

The eggs or larvae are placed in rows on the medium adjacent to shallow grooves made in the medium with the tips of a pair of forceps (Fig. 4). These grooves are necessary to permit the larvae to enter the medium easily.

Larval media

Over 700 different larval media were tested. Many did not permit any larval development. The medium which has given the best results to date is shown in Table II. This medium will be referred to hereafter as "the standard medium".

The medium is prepared by bringing all the ingredients mixed in water to a boil except the hydrochloric acid and the dehydrated carrot. After the mixture has been allowed to cool to about 70°C, the hydrochloric or citric acid is added. This mixture is then homogenized in a high-speed blender for about one minute. The powdered dehydrated carrot is added and stirred in slowly with a mixer. If the dehydrated carrot is homogenized with the

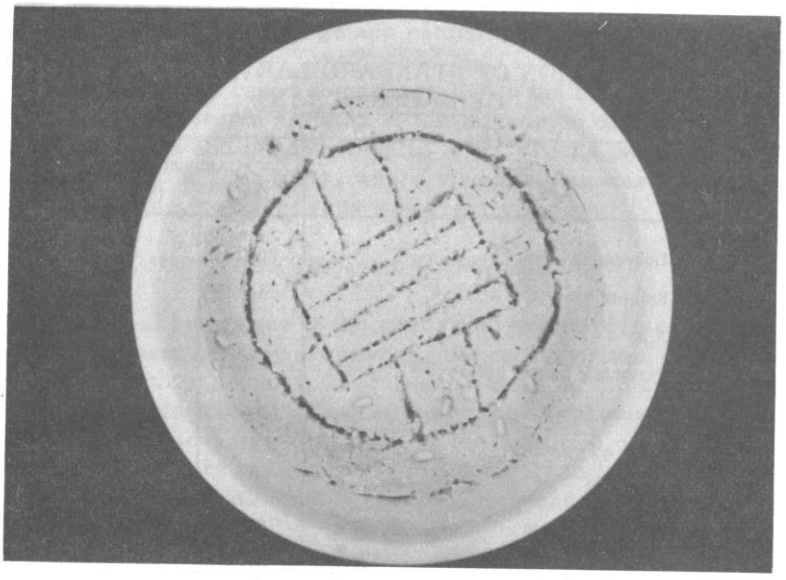

Fig. 4

Puparia of Dacus oleae on surface of larval medium
where they pupated after the larvae came to the surface
The grooves in the medium enable the larvae to enter the medium
after hatching from eggs placed on the flat upper surfaces.

other ingredients the medium becomes so dense that the larvae appear not to be able to utilize it.

If no blender is available, the above procedure should be followed but the mixture should be well stirred when the acid is added, and the carrot powder should be stirred in afterwards until a smooth consistency is obtained. The pH can be easily ascertained fairly closely by using indicator paper.

The media were tested in various containers. The container found most convenient for most experiments was a thin plastic dish (Fig. 4) 9 cm in diameter at the top and 4 cm deep. About 50 ml of medium was poured into the dish. The depth of the medium was usually around 1 cm. Three such dishes were used for each test medium. The medium must not be allowed to dry, therefore another larger-diameter plastic dish was placed tightly over the medium dish.

The larvae enter the medium and disappear. Very little activity is observed until a day or two before pupation. At this time large holes begin to appear in the surface of the medium, and outlines of galleries can be seen through the thin sides and bottom of the dish. The larvae come to the surface of the medium to pupate.

The first larvae complete their development usually 10-13 d at 25°C on the better media. On the first or second day following the first puparia product, the peak number pupate, and within five days from the appearance of the first puparia all larvae have usually completed their development. The speed of larval development is an indication of the nutritional quality of the medium.

TABLE II

COMPOSITION OF STANDARD LARVAL MEDIUM OF DACUS OLEAE

Substance	Amount (g)	Amount (ml)
Dehydrated carrot 1/	25.0	
Brewers' yeast	15.0	
Agar	2.0	
Enzymatic protein hydrolysate of soya 2/	6.0	
Choline chloride	0.05	
Olive oil (virgin)		15.0
Tween 80		5.0
Sodium benzoate	0.3	
Butoben 3/	0.05	
2 N hydrochloric acid 4/		7.0
Water		125.0
	48.4	152.0

1/ Puccinelli Packing Co., Turlock, California.
2/ Nutritional Biochemicals Corp., Cleveland, Ohio.
3/ n-butyl parahydroxybenzoate (Merck).
4/ Enough acid to adjust the medium to a pH reaction of about 4.1.

Puparia handling

The puparia are collected daily from the medium and placed in Petri dishes along with a small wad of water-soaked cotton-wool. This added moisture is important to normal pupation during the first few days after formation.

The weight of the puparia is also another index as to the nutritional quality of the medium. Since the puparial weight may vary with age, one-day-old puparia were weighed and recorded. It appears that the larger puparia in any one test are the first ones produced.

The number of puparia recovered versus the number of eggs that hatched on the medium or the number of larvae placed on the medium does not necessarily indicate nutritional quality, for it is believed that the physical nature of the media, and the method of egg handling at the time of placement, influence survival.

The greatest mortality occurs during the first larval instar. Suffocation due to oil blocking the tunnels may account for most mortality. Dacus oleae

larvae do not back out of tunnels, whereas some other trypetids living in rather fluid media can submerge and return their spiracles to the surface for air [34].

Alien bacteria may cause some mortality in the first-instar larvae, for great numbers of bacteria situated at the micropyle of the egg might gain entry into the larva and be detrimental to it. These bacteria are not the normal symbiote. In later tests, where streptomycin was fed to the adults, the bacterial contamination of eggs was greatly reduced.

RESULTS AND OBSERVATIONS REGARDING ADULTS

The biological responses to the adults that are important to culture and can be observed extrinsically are mating, the preoviposition period, fecundity, fertility and longevity. Some data concerning these functions are from adults reared from puparia from field-collected olives (Figs. 5 and 6). The other data are from flies that were produced on the artificial media.

Fecundity, fertility and longevity are not only influenced by adult diet, but may also be greatly affected by larval diets and uncontrolled bacterial activity in the adult.

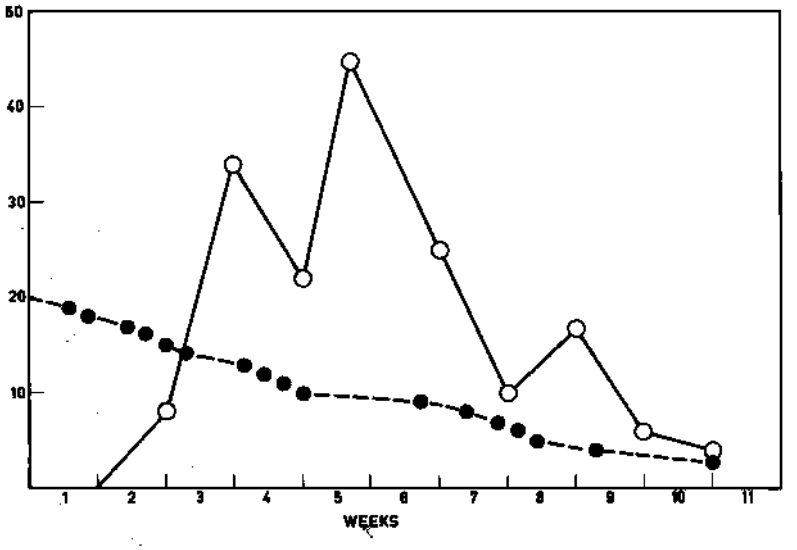

Fig. 5

Average fecundity per Dacus oleae female per week and longevity of 20 females reared on olives

○ No. of eggs per female per week
● No. of living females

Mating

Experiments showed that the males will mate effectively if fed only carbohydrate solutions. In this respect, D. oleae is unlike D. dorsalis [31], for in the latter species both sexes must ingest protein in their adult diet in

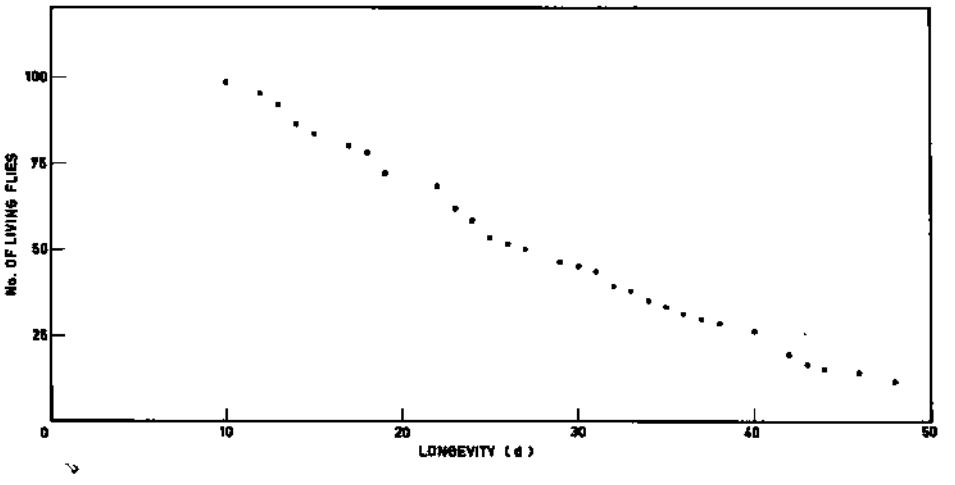

Fig. 6

Longevity of 100 Dacus oleae adults that emerged from field-collected puparia and fed on yeast hydrolysate + sucrose + choline chloride + water

order to copulate. The male of D. dorsalis shows viable spermatozoa in the testes early but will not mate at any age until it ingests protein and requires at least five days after being exposed to the protein before it will attempt to copulate.

The males of D. oleae usually begin mating in four days after emergence, but initial mating on the part of the male may occur earlier or later depending upon the attraction of the female. Long preoviposition periods are correlated with correspondingly late initial matings. Mating is apparently triggered by decreasing light intensities associated with the approach of the twilight period. This mating response to light-change seems to be the same as that reported for D. dorsalis [37] and D. tryoni. Thus it is important that D. oleae be kept in rooms where natural diurnal light is available.

A perceptible sound or call is made by the male in the approach to a susceptible female [35]; this occurs also in other Trypetidae [36]. There can be many matings by the same individuals of D. oleae during their life. The frequency of remating is greater in D. oleae than in the five other trypetids now being mass-cultured. Only during the second mating attempt, which occurred on the second evening following the first mating, was there any reluctance to accept an approaching male. However, during each successive evening for 10 days mating could be observed. It was not determined whether any spermatozoa were transferred after the first mating.

Preoviposition period

The length of this period can vary considerably. The season of the year, the nutrition of the larvae and the nutrition of the adult influence the length of the preoviposition period.

During November, December and January the preoviposition period was the longest in the laboratory, where the temperature was more or less

TABLE III

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF ENZYMATIC PROTEIN HYDROLYSATE OF SOYA[1] UPON THE DEVELOPMENTAL PERIOD OF THE LARVAE, THE WEIGHT AND RECOVERY OF PUPARIA AND THE PREOVIPOSITION PERIOD OF ADULTS

Concn soya hydrolysate[2] (g)	No. fertile eggs[3]	Minimum period to pupation (d)	Wt. of puparia (mg)		Puparia recovered (%)	Fly emergence (%)	Minimum preoviposition period (d)
			Mean	Range			
5	76	13	6.1	5.9-6.3	39	100	23
6	87	11	6.2	5.7-7.0	32	100	13
7	79	11	6.0	5.7-6.4	20	100	13
8	99	11	6.1	5.7-6.7	45	100	13

1/ A product of the Nutritional Biochemicals Corp., Cleveland, Ohio, USA.
2/ Other ingredients as shown in the standard medium (Table II).
3/ Number of eggs that hatched on the medium.

TABLE IV

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF ENZYMATIC PROTEIN HYDROLYSATE OF CASEIN[1/] UPON THE DEVELOPMENTAL PERIOD OF THE LARVAE, THE WEIGHT OF THE PUPARIA AND THE PREOVIPOSITION PERIOD OF ADULTS

Concn[2/] casein hydrolysate (g)	Amount[2/] 2N HCl used (ml)	No. fertile[3/] eggs	Minimum period to pupation (d)	Wt. of Puparia (mg)		Puparia recovered (%)	Fly emergence (%)	Minimum preoviposition period (d)
				Mean	Range			
2	6	95	12	6.1	5.4-6.4	25	100	35
4	7	75	11	6.5	6.4-7.0	30	100	17
6	7	87	10	6.1	6.0-6.2	25	100	16
8	7	100	10	6.0	5.7-6.2	33	100	-
8	8	98	9	6.1	5.9-6.2	21	100	16

1/ Product of the Nutritional Biochemicals Corp., Cleveland, Ohio, USA.
2/ Other ingredients as shown in standard medium (Table II), but casein hydrolysate used in place of soya hydrolysate.
3/ Number of eggs that hatched on the medium.

constant. Either the photoperiod or the amount of light intensity per day may be involved. Perhaps the light intensity influences the feeding rate.

The data shown in Tables III and IV indicate that the concentration of protein hydrolysate in the larval medium may influence the adult preoviposition period. The higher protein hydrolysate concentrations are correlated with shorter preoviposition periods. Thus in D. oleae there is definitely metabolite transfer from larval feeding that influences egg production.

Adult nutrition has an important influence on the preoviposition period in other trypetids [31, 32], and in D. oleae some studies have been made by MOORE [33].

Fecundity

The number of eggs produced by D. oleae is apparently less than in the five species now being mass-cultured. The fact that only one egg is deposited per oviposition shows the olive fly to be strikingly different from the other species cultured, which deposit about 20-60 eggs per deposition. Even though the olive fly appears to live longer than the other species, its total fecundity does not seem to approach that of the other five species. A rough comparison would be of the order of 300:2000 under laboratory conditions. MOORE [33] obtained as many as 700 from some individuals of D. oleae.

The data shown in Fig. 5 are the number of eggs deposited per female per week by adults reared from olives. The average total number of eggs deposited per female was 183, the range being 99-276. In this case the flies were held in small 15-cm-cube cages.

An average of about one egg per female per day is obtained from the larger stock cages (30 cm cube) as compared with 2.5 eggs per female per day obtained over a 10-wk period. The proximity of the female to the feeding and ovipositional sites may account for the increased fecundity, and there is less interaction between flies in the small cages.

The presence of an alien species of bacteria in the morphological sites of the normal symbiote can greatly influence fecundity by shortening longevity. What appears to be the same bacterial species is in some individuals under control while in others the increase is so great in the bacterial pockets of the ovipositor normally occupied by P. savastanoi that blockage of the ovipositor occurs, which prevents both oviposition and excretion and results in the premature death of the female. A typical symptom of this condition is manifested by an extruded ovipositor which cannot be retracted. Pressing the tip of the extruded ovipositor on a microscope slide will reveal great masses of bacteria. There is no general septicaemia in the adult at the time of death. The influence of the pathogenic condition in the female on egg production is shown in Fig. 7.

Five generations of D. oleae were cultured in the presence of what was apparently the same species of bacteria. These bacteria accounted for erratic fecundity as between individuals. The variability in pathogenicity may be a reflection of the density of bacteria that made an original entry into the larvae from the egg, for the number of bacteria surrounding the micropyle of the egg would vary greatly. However, since no identification

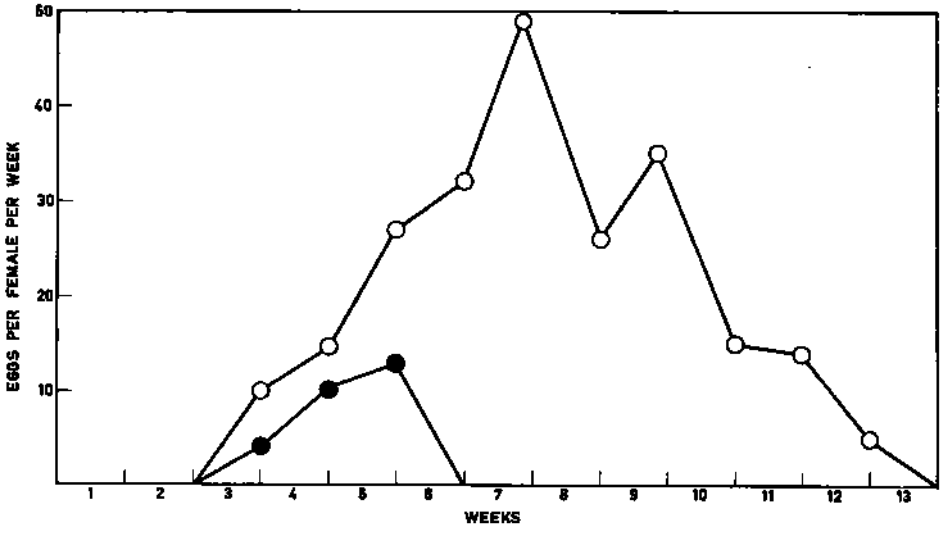

Fig. 7

Average fecundity per Dacus oleae female per week
for flies cultured on the standard medium,
except that 5 g enzymatic protein hydrolysate was used

○ Without pathogenic bacteria
● With pathogenic bacteria

The sample population consisted of 13 flies of which 5 were observed
to be infected with pathogenic bacteria and 8 were not infected.

of the bacteria has been made it may be that several species are involved, including Agrobacterium luteum, which is known to occur in D. oleae [27, 29].

It was thought that perhaps the bacterial species that replaced P. savastanoi under our culture conditions was necessary, but the inclusion of streptomycin in the adult diet has increased fecundity by extending longevity. Two generations have been cultured with streptomycin in the adult diet. Thus it now appears that D. oleae can be cultured without any bacteria present in the morphological sites normally occupied by Pseudomonas savastanoi.

Longevity

The longevity of D. oleae is quite variable, as shown in Figs. 5 and 6. The data shown in these graphs are based on adults reared from olives; thus the normal symbiote was present and should give a more natural picture of mortality. The female mortality shown in Fig. 5 seems unusually high for the first week as compared with the mortality occurring with 100 flies, shown in Fig. 6. The history of these two lots of flies is somewhat different with the 20 females emerging from laboratory-infested olives and the lot of 100 from field-collected puparia. However, the former flies were fed a yeast hydrolysate plus sucrose diet without choline chloride. Males tend to live longer than females.

RESULTS FROM LARVAL MEDIA

The xenic approach in trying to culture D. oleae under non-aseptic conditions, testing all the known media that have been used to culture the five trypetid species mentioned in the introduction, failed to permit larval development. These media, most of which have a carrot base, are rather fluid in nature, and D. oleae larvae would drown. The few known agar, casein and yeast media also gave poor results, as shown in Tables V and VI.

Protein

Poor results were obtained from all media tested until an enzymatic protein hydrolysate was incorporated in the media. Some larvae completed their development on media with high brewer's yeast concentrations, and where casein was included (Tables V and VI), but no oviposition was obtained from the resulting adults.

At first, yeast extract was used in combination with the protein hydrolysates, but better results were obtained with higher levels of brewer's yeast (Table VI, Fig. 8). By varying the protein source the speed of larval development was influenced as well as the size of puparia produced.

The two enzymatic protein hydrolysates that gave the best results were those of soya (Table III, Fig. 10) and casein (Table IV, Fig. 11). An enzymatic protein hydrolysate of casein permits the most rapid larval development of all the protein sources tested, nine days being the shortest period observed. An effective concentration of casein hydrolysate is about 2%. This concentration can be used in place of the 3% soya hydrolysate used in the standard

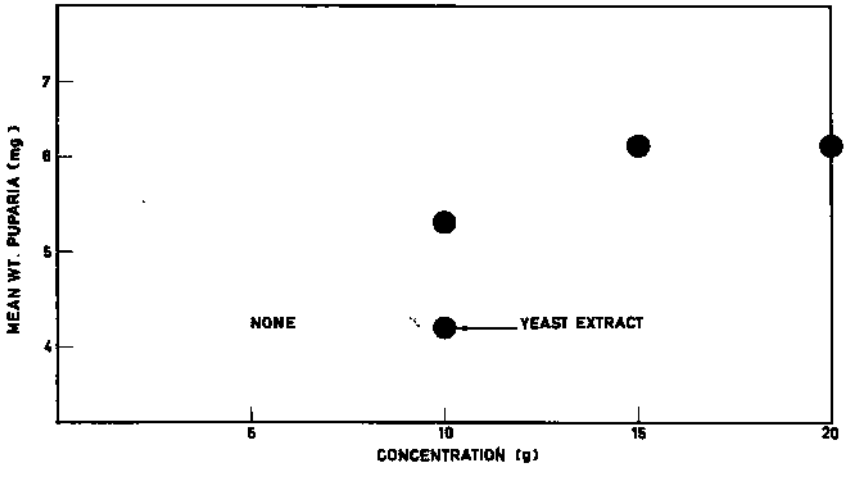

Fig. 8

Relationship between concentration of brewer's yeast
and one comparable yeast extract in the standard larval medium
and weight of Dacus oleae puparia produced
All media contained 5 g enzymatic protein hydrolysate of soya.

TABLE V

INFLUENCE OF SOME DIFFERENT CONCENTRATIONS OF BREWER'S YEAST IN BASAL MEDIA WITHOUT ADDITIONAL PROTEIN (EXCEPT FROM DEHYDRATED CARROT) UPON THE WEIGHT AND RECOVERY OF PUPARIA AND THE REPRODUCTION OF THE ADULTS PRODUCED

Brewer's yeast concn (g)	Sodium benzoate concn (g)	Citric acid (g) or HCl (ml)	No. fertile eggs	Minimum period to pupation (d)	Wt. puparia (mg)		Puparia (%)		Visible contamination	Fly emergence (%)	Production of fertile eggs
					Mean	Range	Mean	Range			
10	0.3	5								None	
15	0.3	1.5	100	18	4.1	2.0-5.9	9	5-13	Some	13	None
20	0.3	5	67	17	5.3	5.0-5.3	3	0-4	Some	50	None
20	0.35	1.5	44	16	6.2	5.5-7.5	10	6-14	Some	50	None
25	0.3	1.5	37	16	3.6	2.9-4.3	5	0-6	Heavy	None	
30	0.3	1.5	80		None				Heavy	None	
30[1]	0.3	1.0	60		None				Heavy	None	
40[2]	0.3	1.5	37	15	6.2	4.5-8.8	10	0-14	Heavy	None	

1) Without dehydrated carrot
2) With 9% dehydrated carrot instead of standard 15%.

TABLE VI

INFLUENCE OF SOME DIFFERENT PROTEIN SOURCES AND THEIR STATE OF HYDROLYSIS UPON THE DEVELOPMENT OF DACUS OLEAE LARVAE, PUPARIA AND ADULTS

Protein source	Type of hydrolysate	Concn (g)	Brewer's yeast concn (g)	Brewer's yeast extract concn (g)	No. fertile eggs	Minimum period to pupation (d)	Wt. puparia (mg)		Puparia recovered (%)		Visible contamination	Fly emergence (%)	Production of fertile eggs
							Mean	Range	Mean	Range			
Soya	Not hydrolysed	5	15	–	120		None						
Soya	" "	8	--	10	75		None						
Soya	Papain	5	--	10	185	12	3.7	2.7-5.4	33	24-45	None	90	yes
Soya	"	5	--	15	38	12	4.9	4.2-6.0	35	30-41	None	100	yes
Soya	"	8	--	10	100	12	5.2	4.3-6.6	11	10-14	None	80	yes
Soya	"	5	15	--	100	12	6.1	4.9-8.3	42	32-50	None	100	yes
Soya	"	5	20	--	50	12	6.0	5.2-7.8	55	35-62	Some	90	yes
Soya	"	8	20	--	65	12	6.0	4.9-8.1	21	16-26	Some	100	yes
Soya	"	10	20	--	30	12	6.7	5.6-7.7	44	35-62	Some	100	yes
Soya	Trypsin	5	--	10	50	16	3.7	2.9-4.9	18	18-18	Heavy	70	None
Soya + Casein	Papain Trypsin	5	15	--	50	13	5.3	4.0-7.0	22	0-44	None to Heavy	100	yes
Casein	Not hydrolysed	8	--	10	100		None						
Casein	" "	8	--	10	55	13	4.4	3.9-4.9	3	3-3	None	50	None
Yeast	Trypsin	5	15	--	100	13	5.5	4.2-7.0	19	12-33	Some	80	None
Mean	Proteose peptone	5	--	10	40	15	4.9	4.6-5.2	5	0-8	Heavy	None	

1) Other ingredients in media, such as dehydrated carrot, agar, olive oil, Tween 80, choline chloride, sodium benzoate and pH, are at the same concentrations. The temperature was 25 ± 1°C; the relative humidity was 70 ± 20%.

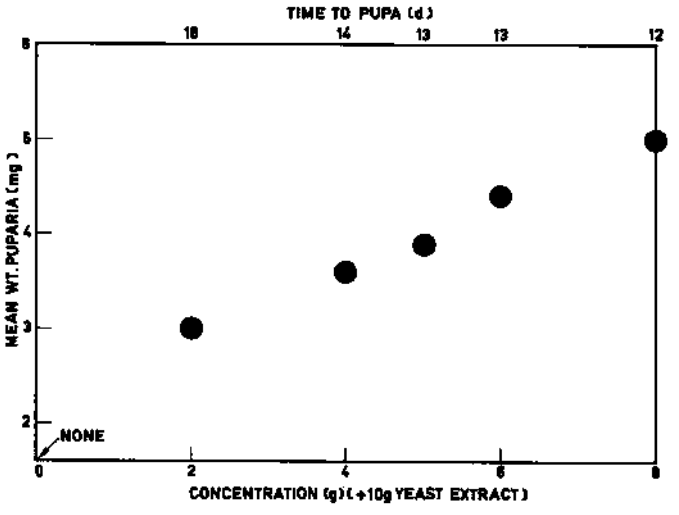

Fig. 9

Relationship between concentration of enzymatic protein hydrolysate of soya in the standard larval medium, weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage
10 g yeast extract used in place of 15 g brewer's yeast in the standard medium.

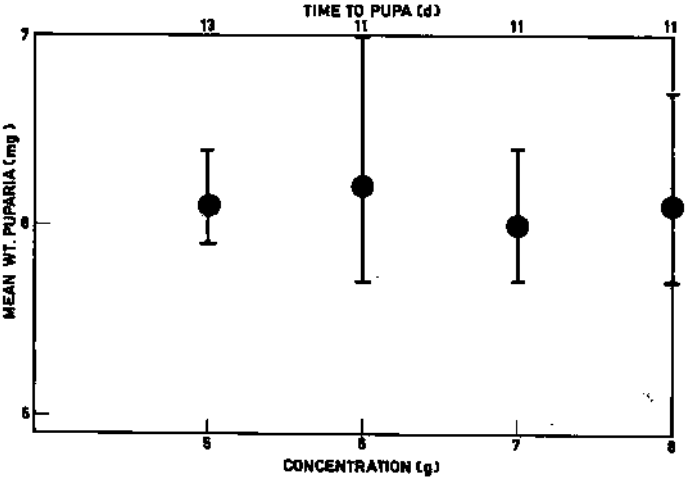

Fig. 10

Relationship between concentration of enzymatic protein hydrolysate of soya in the standard larval medium, mean weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage

diet. Enzymatic protein hydrolysates such as lactalbumin, proteose peptone, bacto-peptone and yeast gave poor results.

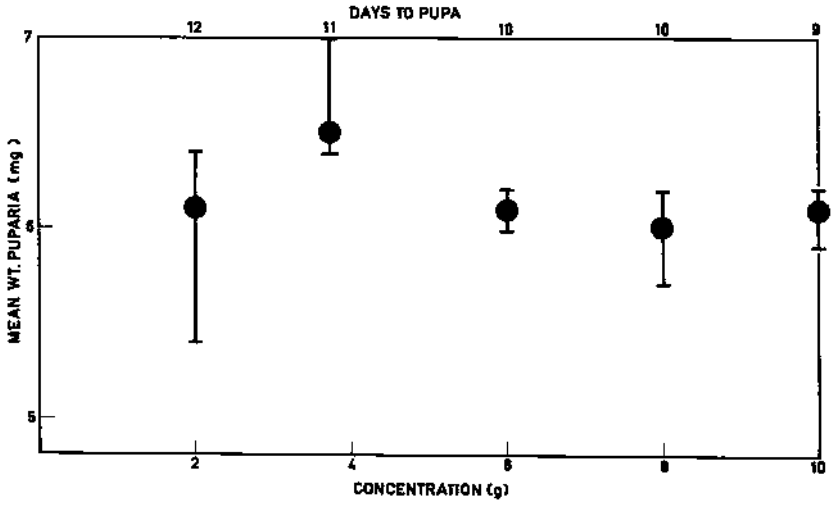

Fig. 11

Relationship between concentration of enzymatic protein hydrolysate of casein in the standard larval medium, mean weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage

Unhydrolysed protein sources used in the standard diet in place of hydrolysed protein such as casein, egg albumin, soya meal and dehydrated skim milk permitted none or less than 5% of the larvae to develop, and no reproduction occurred.

Carrot

Fresh carrot blended and supplemented with all the other ingredients outlined in Table II gave poor results and became rather heavily contaminated.

Dehydrated carrot from California and France*

Reduced levels of carrot permit development, but puparial emergence is reduced and adults are weak.

Brewer's yeast

Relatively high concentrations of brewer's yeast are necessary as compared with the other trypetids cultured A level of about 7% has given the best results when a protein hydrolysate is also present in the medium. Brewer's yeast is superior to baker's yeast or yeast extract, even though the biological activity of the latter is considered much higher. The water-insoluble factors present in brewer's yeast seem to be utilized, as indicated by the increased weight attained over that of yeast extract (Table VI, Fig. 8).

* The dehydrated carrot from France that was tested was donated by Dr. M. FERON, Station de zoologie, centre de Recherches Agronomiques du Sud Est, Montfaret, France.

Agar

If eggs are deposited directly into 3-4% agar which has a coating of paraffin, the eggs hatch and the larvae readily tunnel. However, in the standard medium outlined in Table II 1% is adequate. Higher levels do not seem to permit easy entry when the eggs or larvae are placed on the surface.

Olive oil

Although varying the concentration of the olive oil does not seem to influence the weight of the puparia produced or the speed of development of the larvae, the percentage of flies emerging from the puparia is influenced. About 7% olive oil permits 100% emergence and appears to allow the medium to be more extensively used. Tween 80 is necessary for emulsifying the olive oil. Concentrations above 2.5% were detrimental and Tween 40 gave inferior results.

Mould inhibitors

Sodium benzoate, n-butyl parahydroxybenzoate (Merck's Butoben), methyl paraoxybenzoate, sodium propionate and sorbic acid were tried at many different concentrations and combinations. We alternate between using the combination shown above with media containing only 350 mg sodium benzoate. Further testing is still necessary, for the media will still allow some moulds and yeasts to develop. When using protein hydrolysates it is particularly difficult to control microorganisms.

Adjusting the pH factor to about 4.1 is necessary in the attempt to control bacteria. Factors above 4.4 permits heavy contamination. It appears that one larva requires about 2 ml of the medium to develop. The cost of the larval medium is about US $1.25 per 1000, on the basis of obtaining one fly per 2 ml.

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DISCUSSION

H.J. BOROUGH: Is it possible that the colour of the paraffin egg-attracting dome has any effect?

K.S. HAGEN: No specific tests have been made to determine this. Observations with a pale green colour and a paraffin colour did not seem to show much difference. Actually, the addition of 5-10% beeswax to paraffin with a melting point of 52-53°C makes the paraffin a little harder but also alters the colour slightly. The addition of beeswax appears to influence the numbers of eggs deposited. The increase in eggs may be related to colour.

G. SILVA: At what temperature did you rear your pupae? You mentioned that the rearing took nine days, but in Portugal we have never recorded less than twelve days for pupae obtained from naturally infested olives.

K.S. HAGEN: The temperature was 25 ± 1°C. I should say that the figure does not include the incubation period of the egg, which is about 2.5 d at that temperature.

M. FERON: I should like to congratulate you on the work you have accomplished in a relatively short time on such a difficult subject.

You mentioned in your oral presentation that the acidity of the culture medium prevents the development of bacteria. In general that is true, but in the course of our experiments on Dacus we found that there was some bacterial development and our microbiologist, Mr. Vago, informed us that they were acidophilic bacteria.

You also stated that the bacteria which you find replacing the symbiote

may be pathogenic for Dacus. If I remember correctly, this has also been noted in the case of Pseudomonas savastonoi.

As regards the difficulty of obtaining eggs, we made the egg-laying places more attractive to the females by adding a small quantity of olive oil to the paraffin. The females then come to lay more readily, so that the deposition of eggs on the walls of the cage is avoided, but the situation is still not entirely satisfactory and there is no doubt that the problem of obtaining and handling eggs still constitutes a great obstacle to large-scale culture.

It should be possible to determine the reproduction habits of Dacus oleae. We have devoted some time to such a behavioural study, as you know. Our studies bore particularly on sexual behaviour, but I imagine behaviour at oviposition should be largely governed by comparable conditions. The sexual behaviour involves the call of the male (vibration of the wings) followed by mating, and it is more intense at 22°C than at 25°C.

B. BACCETTI: Is it possible to say for certain that the diet of the adult Dacus contains no lipids? I should like to know this in connection with a comparative study of excretion in the larva and the adult.

K.S. HAGEN: No lipid is included in the adult diet separately. There are probably traces of sterol in the enzymatic protein hydrolysate of yeast that is used, even though it appears to be completely water-soluble. The sterol requirement in the adult can be satisfied at least partially by metabolite transfer from larval feeding. The larval medium is rich in sterols and lipids. Choline chloride supplementation in the adult food may in some way play a role in sterol or lipid metabolism.

C. PELEKASSIS: What is the effect of environmental factors, such as light intensity or relative humidity, on the oviposition rate?

K.S. HAGEN: Light intensity or photo-period do seem to have some influence on reproduction. During November and December the preoviposition period is prolonged compared with September or March. The temperature was the same in the laboratory during both periods.

M. FERON: The action of light is certainly very important in the reproduction behaviour of Dacus oleae. We got results comparable to those published for Dacus tryoni in Australia. The main factor is the duration of exposure of the adults to light. After a period of darkness corresponding to night the insects are inactive and sexual activity only commences after exposure to light for about six hours; it is facilitated by a reduction in light intensity (corresponding, under natural conditions, to dusk), but it still appears, with a certain delay, after continuous exposure to a constant light intensity. Several hours of darkness are necessary for the insect to return to a state of complete rest. The intensity of the light also plays a part. It appears that there is an optimal intensity of the order of 400 to 600 lx. These points should be investigated in further experiments.

K.S. HAGEN: What is the mode of action of the light? What is the light doing to the fly to increase this activity?

M. FERON: I think that the light acts through the medium of a hormone. With Dacus, exposure to light could result in the accumulation of a hormone required to initiate the behaviour; once a certain quantity is produced the process is triggered off, while darkness lasting for several hours might result in the elimination of the hormone.

ÉTUDE DES POPULATIONS ET DE DISPERSION DE CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) EN TUNISIE A L'AIDE DES RADIOISOTOPES

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Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPE INVESTIGATION OF CERITATIS CAPITATA WIED. (DIPT. TRYPETIDAE) POPULATION AND DISTRIBUTION IN TUNISIA. Very heavy damage to Tunisian fruit production caused by Ceritatis capitata Wied. is the background to preparations to attempt control by gamma-ray sterilization of the males.

As a follow-up to ecological studies already completed and preliminary distribution tests, P^{32}-labelled flies are being used in a technique worked out for studying populations; its value seems confirmed by results obtained by conventional techniques.

The population estimate made indicates that, in different plantations, there might be 2 to 9 males per hectare by the end of winter.

ÉTUDE DES POPULATIONS ET DE DISPERSION DE CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) EN TUNISIE A L'AIDE DES RADIOISOTOPES. Les très importants dégâts provoqués par Ceratitis capitata Wied. aux productions fruitières tunisiennes sont à l'origine des travaux destinés à préparer la mise en oeuvre de la lutte par stérilisation des mâles aux rayons gamma.

En complément des études écologiques déjà réalisées et des essais préliminaires sur la dispersion, une méthode d'étude des populations a été mise au point, en utilisant des mouches marquées au ^{32}P; la valeur de cette méthode paraît être confirmée par les résultats donnés par d'autres procédés classiques.

L'estimation de population ainsi réalisée a montré qu'il pouvait y avoir de 2 à 9 mâles par hectare à la fin de l'hiver dans différentes plantations.

ИЗУЧЕНИЕ ПОПУЛЯЦИЙ И РАСПРОСТРАНЕНИЯ CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) В ТУНИСЕ С ПОМОЩЬЮ РАДИОИЗОТОПОВ. Весьма значительный ущерб, нанесенный Ceratitis capitata Wied. плодовому производству Туниса, явился причиной проведения работ в целях разработки способов борьбы путем стерилизации мужских особей гамма-лучами.

В дополнение к уже проведенным экологическим исследованиям и предварительным опытам по вопросу о распространении был разработан метод изучения популяций с использованием мух, меченных P^{32}; представляется, что ценность этого метода подтверждается результатами некоторых других обычных способов.

Проведенная таким образом оценка популяций показала, что в конце зимы на одном гектаре различных плантаций может находиться от 2 до 9 мужских особей.

ESTUDIO MEDIANTE RADIOISÓTOPOS DE LAS POBLACIONES Y DE LA DISPERSIÓN DE LA CERATITIS CAPITATA WIED. (DIPT. TRIPÉTIDO) EN TÚNEZ. Las importantes pérdidas que la mosca Ceratitis capitata Wied. causa a la producción frutera de Túnez obligaron a emprender un estudio a fin de preparar los medios para combatirla por esterilización de los machos con rayos gamma.

Para complementar los estudios ecológicos ya realizados y los ensayos preliminares sobre la dispersión, se ha preparado un método de estudio de las poblaciones utilizando moscas marcadas con ^{32}P; el valor de este método ha sido confirmado al parecer por los resultados obtenidos con otros métodos clásicos.

El cálculo de la población así realizado muestra que a fines de invierno se encuentran probablemente de 2 a 9 machos por hectárea.

I. INTRODUCTION

L'économie fruitière de la Tunisie est commandée pour une part importante par le problème de la mouche méditerranéenne des fruits, Ceratitis

capitata Wied., qui empêche toute production fruitière d'été et oblige à de coûteux traitements sur les agrumes. C'est pourquoi l'Institut national de la recherche agronomique de Tunisie consacre un effort particulier à l'étude de ce problème.

Le Colloque d'experts sur la lutte contre les insectes par la méthode des mâles stériles, organisé par l'Agence internationale de l'énergie atomique à Vienne en octobre 1962, examinait les possibilités d'application de cette méthode à la lutte contre Ceratitis dans le bassin méditerranéen; il apparut aux membres du Colloque que la Tunisie présentait des conditions particulièrement favorables et qu'un programme d'action devait être mis en œuvre.

Le premier point du travail devait être de compléter la connaissance écologique de Ceratitis à partir des études déjà réalisées [1 - 5], ce qui a pu être entrepris avec l'aide de l'Agence.

Une prospection d'ensemble a été réalisée grâce à l'heureuse initiative et à l'aide efficace que M. Féron, Directeur de la Station de zoologie agricole de Montfavet, nous a apportées; nous l'en remercions bien vivement ici, car cela a permis d'établir les bases de ce travail écologique et de choisir une zone pilote assez favorable [6].

La connaissance aussi précise que possible de la population de mouches aux différentes époques de l'année et des possibilités de dispersion des mouches lâchées est évidemment à la base de l'application de la méthode de lutte par les lâchers de mâles stériles. C'est dans ce sens que nous avons entrepris ce travail en utilisant, conjointement avec d'autres méthodes, la technique du marquage des insectes par radioisotopes.

II. ESSAIS PRÉLIMINAIRES

En novembre 1960, en collaboration avec un expert de l'AIEA, M. J. F. Cline, un premier essai d'orientation avait été effectué dans l'orangeraie de l'INRAT: sur les 353 mouches marquées à l'aide de ^{32}P, (0,5 mc pour 100 g d'aliment à base de miel, sucre et levure de bière), lâchées au milieu des agrumes, 70, soit 20%, ont été reprises grâce à des gobe-mouches disposés à cet effet et appâtés avec du phosphate bi-ammonique à 2%. La distance maximum, parcourue en trois jours, avait été de 315 m.

L'année suivante, au milieu d'un important verger de pêchers en pleine maturité, 2200 mâles de Ceratitis étaient lâchés. Un réseau de 280 pièges au phosphate bi-ammonique permettait de reprendre 449 mouches, soit 20,4%; la moitié de ces mouches étaient capturées à moins de 100 m du point du lâcher, un quart entre 100 et 200 m et un quart au delà jusqu'à un maximum de 610 m (malgré la présence de haies brise-vents). La rapidité de dispersion atteignit 460 m en 24 h [7].

L'intéressant taux de recapture observé au cours des essais préliminaires et la facilité d'emploi du ^{32}P pour le marquage des mouches nous engageait à poursuivre les travaux entrepris.

De ce fait, un programme fut mis sur pied en 1962, destiné à fournir les renseignements de base nécessaires à une future éradication de Ceratitis en Tunisie, utilisant la méthode de la stérilisation des mâles à l'aide des rayons gamma.

Ce programme a pu entrer en action grâce à l'AIEA qui a apporté une contribution financière importante et la compétence d'un de ses experts, M. Jurenka, qui nous a utilement guidé dans la mise au point de la technique de marquage.

III. TECHNIQUE DE MARQUAGE ET DE LÂCHER

Afin de préciser la forme de ^{32}P et les doses à adopter pour notre étude, un marquage au laboratoire a été expérimenté, permettant de comparer la forme acide PO_4H_3, la forme neutre PO_4HNa_2 et le phosphore rouge irradié (91 h d'irradiation).

Le phosphore rouge fut tout de suite écarté, du fait qu'il se présentait en poudre très peu soluble.

Des formes acide et neutre, la première fut choisie pour la suite des travaux en raison de sa bonne efficacité et de son prix moins élevé; la concentration fut fixée à 1 mc pour 100 g d'eau sucrée à 10%.

Le type de boîte de lâcher, mis au point au laboratoire et qui s'est révélé à l'expérience être fort bien adapté à notre travail, permet de contaminer 100-200 mouches à la fois; à cet effet, 20 cc de solution radioactive imbibent une éponge artificielle disposée au fond de la boîte. Celle-ci peut être accrochée à une branche et son couvercle levé à distance grâce à un long fil de nylon, ceci pour éviter que les mouches contaminées ne viennent aussitôt au contact de l'épiderme (suivant une réaction assez fréquente).

IV. ESTIMATION DE POPULATION ET DE DISPERSION DANS UNE ORANGERAIE

Un essai fut réalisé dans une orangeraie proche du laboratoire pour tester l'efficacité de la technique et si possible pour en tirer quelques indications sur la présence de Ceratitis à la fin d'un hiver particulièrement rigoureux.

Deux mille mouches, en 10 boîtes de lâcher de 200 mouches, à raison de 1 boîte pour 4 arbres, furent lâchées à la fin mars de cette année au centre d'une orangeraie de 8 ha. Le temps était incertain, avec beaucoup de vent, et la pluie se déclencha au moment où étaient libérées les dernières mouches. La pluie, assez forte, dura 1 ha. Puis un vent soutenu assécha le sol sablonneux.

Les boîtes de lâcher, laissées ouvertes pendant 6 h, ne contenaient plus au total que 127 mouches mortes ou inactives. Près de 400 pièges en plastique étaient alors disposés sur toute l'orangeraie, à raison de 1 pour 4 arbres; l'attractif utilisé était le medlure, suivant la méthode américaine [8].

Le lendemain, 28 de ces pièges avaient repris 125 mouches, toutes fortement contaminées. La dispersion, avec le mauvais temps, s'avéra très faible. Trois jours après, 126 autres mouches étaient reprises, dont 2 non marquées.

Le taux de recapture a donc été de 13,3%; si l'on compte les 2 mouches non marquées comme étant de la population naturelle, cela nous permet

d'estimer, d'après l'indice de Lincoln [9], cette population naturelle comme étant de 15 mâles pour les 8 ha de l'orangeraie, soit peut-être une trentaine de mouches mâles et femelles, si le sex-ratio est de 1; c'est-à-dire 4 mouches par hectare.

V. ÉTUDE DE POPULATION ET DE DISPERSION DANS UNE ZONE PILOTE - PREMIERS RÉSULTATS

La zone pilote choisie pour les premiers essais de lutte par lâchers de mâles stériles présente des caractéristiques géographiques fort intéressantes. En effet, cette zone est isolée entre la mer et une région de collines arides s'élevant de 200 à 300 m. Elle comprend des vergers composés d'essences diverses constituant un foyer permanent typique pour Ceratitis.

Les vergers choisis pour l'expérimentation comprennent surtout des abricotiers et des figuiers, hôtes de choix de la Trypétide. Des brise-vents cloisonnent assez étroitement les parcelles, offrant à la mouche un biotope très favorable.

Un premier lâcher a été réalisé au début d'avril. Les mouches marquées ont été lâchées dans un secteur de 1 ha environ, leur nombre étant calculé à raison de 1 pour 5 m^2 de terrain, compte tenu d'une mortalité estimée à 6% d'après les précédents essais. Ces mouches, au total 1843, étaient réparties dans 19 boîtes placées dans 11 parcelles du secteur.

Le temps étant ensoleillé, les mouches sortaient rapidement des boîtes (la mortalité fut de 7,6%).

Le dispositif de recapture comprenait 300 pièges secs à raison de 1 par arbre sur la totalité du secteur (1 ha). Les recaptures ont été en 24 h de 425 mouches, soit 23% plus seulement 2 non marquées. Ceci permet d'estimer la population naturelle à 9 mâles environ par hectare (soit peut-être une vingtaine de mouches mâles et femelles).

Ce chiffre faible serait normal à la fin de l'hiver; il est confirmé d'une part par l'essai effectué deux semaines auparavant à l'orangeraie de l'INRAT, même si ce verger se trouve à 50 km de la zone pilote; d'autre part, l'étude de population en cours dans la zone pilote est contrôlée par d'autres recherches, conduites par des méthodes différentes:

En effet, depuis janvier 1963, une centaine de gobe-mouches appâtés aux protéolysats à la dose de 7% ont été installés sur diverses espèces fruitières, assez éloignées de la zone du lâcher; or, en 3 mois de relevés hebdomadaires, seules 3 mouches ont été capturées;

D'autre part, des traitements de choc appliqués mensuellement sur 4 bigaradiers (production d'hiver), 4 abricotiers (production de printemps) et 4 figuiers(production d'été) n'ont encore pas révélé la présence de Ceratitis au cours de l'hiver; il est vrai qu'il ne s'agit que de 12 arbres, alors que le dispositif de recapture des mouches marquées comprend 300 arbres, chacun muni d'un piège;

En hiver également, le ramassage des fruits tombés sous 10 arbres fruitiers, hôtes de Ceratitis à cette époque (brigaradiers), a seul permis de révéler une infestation. Les fruits ramassés sous les 10 bigaradiers,

puis placés en observation en laboratoire, chauffé en hiver, ont donné jusqu'à la mi-mars, 146 larves.

Par ailleurs, le pouvoir de dispersion des mouches marquées à partir du point de lâcher a pu faire l'objet de deux contrôles.

a) D'une part, les résultats du piégeage dans les 300 arbres de la zone de lâcher ont montré une répartition assez remarquablement homogène des captures par arbre; les mouches, en effet, se répartissent après quelques heures sur l'étendue de chacune des parcelles.

b) D'autre part, des barrages constitués par 180 pièges secs ont été disposés à des distances de 500 à 1000 m autour de la zone de lâcher 2 jours après le lâcher et laissés en place pendant 3 jours. Dans trois de ces pièges seulement, situés à environ 700 m du lâcher, 4 Ceratitis mâles ont été capturés; examinés au G-M, ils présentaient une contamination faible, mais nette (3 fois le bruit de fond); il s'agissait donc de mouches marquées.

VI. CONCLUSION

Cette première série d'opérations a permis de mettre au point les techniques de marquage, de lâcher et de capture de Ceratitis dans les conditions rencontrées en Tunisie. Les résultats obtenus dans des biotopes différents, contrôlés par deux autres méthodes, montrent entre eux des similitudes qui confirment la valeur de la méthode du marquage radioactif pour des études de population et de dispersion.

D'un point de vue plus immédiat, il apparaît que la population de mouches, au cours de l'hiver 1963 dans des foyers permanents de Ceratitis, se situe à un niveau faible qui serait de l'ordre de 2 à 9 mâles par hectare. Cette dernière donnée est fondamentale si l'on veut envisager l'application de la méthode de lutte par des lâchers de mâles stériles.

Il est évident que ces premiers résultats n'ont qu'une valeur indicative et qu'ils constituent le début d'un travail écologique qui sera poursuivi tout au long de l'année de façon à bien connaître ces variations de population de Ceratitis dans les foyers permanents.

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DISCUSSION

M. FERON: The work done in Tunisia by Mr. Soria is really very interesting and important. Some of the best experts in the sterile-male technique attended the meeting the Agency organized in Vienna last October*, and I remember in particular that Dr. Lindquist, who is responsible for its application against the screw-worm, laid great emphasis on the need for carrying out very detailed ecological studies beforehand. Dr. Lindquist recalled that in the case of the screw-worm such studies had started before the War and an extremely accurate ecological study had a vital part in the success of the operation. He pointed out that, in general, the real number of insects in a given biotope was inadequately known and entomologists could expect surprises, but usually agreeable ones, i.e. there were far fewer insects than was expected. Observations are, of course, usually made in places where the insects accumulate. Ceratitis can be found in large numbers in an apricot plantation when the fruit is ripening, but then there will probably be few in neighbouring plantations where no such fruit is to be found. This is very important to know, as the vital information is the number of insects per hectare in a given area.

The pilot zone study referred to by Mr. Soria should therefore be continued, certainly for a year, and very probably for a second year, before it can be exactly known where the sterile males should be released. To devise a satisfactory method of releasing, tests can of course be made in smaller areas.

I have one comment on Mr. Soria's results. It is obviously taking a risk to extrapolate a population estimate on a figure of 2 non-labelled flies out of 425 recaptured. There are fortunately complementary methods which validate these figures and show that the real population is extremely small, so that the figures of 2 to 9 males per hectare are no doubt correct. What is important is to have some means of following the population gradient which takes place in about May, and the regression which takes place in autumn.

Another difficulty in estimating the total population is the real sex ratio, the real proportion of males to females. Your calculation assumes that they are equal in number, but this is very difficult to confirm. We know from breeding experience that the males of Ceratitis are more delicate and stand up less well to unfavourable conditions. The same may be true under natural conditions. Here, what we are interested in is the actual number of males, since our aim is to apply the sterile-male technique; the female population is less important, the whole point being that there should be more sterile males than normal males.

You have put one trap per tree throughout the release area; I think one could afford to be more economical in future. Your results show that the

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* Panel on insect population control by the sterile-male technique.

flies spread evenly over the whole of the test area in a few hours, so that a statistically evaluated sampling procedure should be sufficient. This should make it possible to simplify your arrangements and so facilitate the ecological work.

There is one unknown factor which you are very well aware of, i.e. you do not know the duration of life of the males released. For example, if they live longer than a month and you make monthly population studies, your conclusions may be thrown out.

F. SORIA: This will be seen at the next release.

M. FERON: That is quite true. Another aspect of the sterile-male programme which must be considered at the same time is that of devising irradiation methods in order to obtain very large numbers of sterile males. Fairly accurate information is already available from the work done by the Americans in Hawaii and this provides an order of magnitude, but it must be adapted to our conditions and to the biological material we are dealing with; their insect strains are necessarily different from what we have in the Mediterranean, and all the dosimetry and biology work must certainly be checked before we can safely release both males and the females which it would be dangerous to release if they also were not rendered sterile.

There is one remark I should like to make on our own recent work. We unfortunately did not find a method of separating females from males before the insects hatched out, and we tried to find if there was a difference at the pupal stage. There is in fact a difference in weight but also unfortunately a difference in volume. On the average the female pupae weigh 0.5 mg more than the males. This is very little and the weight graphs for the two sexes are so similar that no automatic mechanical way of separating the pupae can be seen at the moment. We also made some experiments with the eggs and were no more successful; we could not find a difference in the relative density of the eggs containing males and females.

Hence if they are to be separated, attractants must be used; otherwise the risk must be taken of releasing females at the same time as males, having first made absolutely sure that the females are sterile.

F. SORIA: I should like to thank Mr. Feron for his advice and very welcome suggestions. Now that the work has become routine in Tunisia we must begin to plan the follow-up. It would be impossible to go directly from the ecological study to the stage of making releases without knowing how the Tunisian flies will react to gamma-ray treatment. Accordingly a great deal of laboratory work must first be done. Once the doses have been determined small releases can be made in limited areas - a small-holding, an isolated orchard in a cereals area, or one of our many oases.

VI.

RADIATION STUDIES: SPECIFIC EFFECTS

EFFECTS OF RADIATION ON GERM CELLS OF INSECTS: DOMINANT LETHALS, GAMETE INACTIVATION AND GONIAL-CELL KILLING

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF RADIATION ON GERM CELLS OF INSECTS: DOMINANT LETHALS, GAMETE INACTIVATION AND GONIAL-CELL KILLING. Radiations and chemical mutagens kill cells in numerous ways: by one of several kinds of induced dominant lethality, by a direct inactivation of function as with sperm, and by genetically undefinable types of death which may or may not be related to dominant lethality per se. Also, chemical mutagens appear to exert a curious enhancement of the fertilizing capacity of sperm.

The different stages of oögenesis and spermatogenesis respond with unequal sensitivity to radiation, and individual cells pass through stages conferring as much as a 50-fold difference in sensitivity.

Where species of Diptera, Hymenoptera and Coleoptera can be compared, a striking similarity of response to radiation can be observed, both to stage sensitivity and degree of response with dose. The silkworm, Bombyx mori (Lepidoptera), seems to be similar in most respects to representatives of the other orders in response of germ cells to radiation, but differs sharply in types of dominant lethality induced. Species having atypical genetic mechanisms (e.g., the lecanoid system of Planococcus citri (Hemipt.: Coccidae) are special cases, and their responses to radiation are considerably modified from those of other species.

For insect population control by the irradiation-of-male method, dominant lethality is as advantageous in species where matings are multiple as in species where mating occurs once. Sperm inactivation and gonial killing can be regarded as instances of true sterility and are maximally effective only in species where mating occurs once. For most efficient control, doses should be chosen which would induce maximum dominant lethality, minimum sperm inactivation and complete killing of gonial cells. These parameters are simple to determine by gamete viability measurements, irradiated and unirradiated population competition experiments and histological examination of gonia.

EFFETS DES RAYONNEMENTS SUR LES CELLULES GERMINALES DES INSECTES: LETALITÉ DOMINANTE, INACTIVATION DES GAMÈTES ET DESTRUCTION DES CELLULES DES GONADES. Les rayonnements et les agents chimiques de mutation détruisent les cellules de nombreuses façons: par l'un des modes d'induction d'une létalité dominante, par l'inactivation directe d'une fonction et par des manières génétiquement indéfinissable, liées ou non à la létalité dominante. Les agents chimiques de mutation paraissent également provoquer un accroissement curieux du pouvoir fertilisant du sperme.

La radiosensibilité varie avec les divers stades de l'oogenèse et de la spermatogenèse; certaines cellules passent par des stades au cours desquels leur radiosensibilité peut varier dans un rapport de 1 à 50.

Des comparaisons entre des diptètes, des hyménoptères et des coléoptères ont révélé une similarité frappante dans la manière dont ces espèces réagissent aux rayonnements, tant en ce qui concerne la sensibilité aux divers stades de développement que l'intensité de la réaction en fonction de la dose. Le ver à soie Bombyx mori (lépidoptère) semble avoir beaucoup de points communs avec les représentants des autres ordres en ce qui concerne l'effet des rayonnements sur les cellules germinales, mais il en diffère nettement pour ce qui est des facteurs létaux dominants induits. Les espèces présentant des mécanismes génétiques anormaux (par exemple, le Planococcus citri, coccidé hémiptère) constituent des cas particuliers et ils réagissent aux rayonnements de façon très différente des autres espèces.

Dans la lutte contre les insectes par la méthode de l'irradiation des mâles, la létalité dominante est aussi efficace pour les espèces où l'accouplement a lieu plusieurs fois que pour celles où il a lieu une seule fois. On peut considérer l'inactivation du sperme et la destruction des cellules des gonades comme des facteurs

de stérilité réelle; leur efficacité est maximum chez les espèces où l'accouplement n'a lieu qu'une seule fois. Pour le contrôle le plus efficace, il faudrait avoir recours à des doses provoquant la létalité dominante maximum, l'inactivation minimum du sperme et la destruction totale des cellules des gonades. Il est facile de déterminer ces paramètres en calculant la viabilité des gamètes, au moyen d'expériences comparatives avec des populations non irradiées ainsi que par l'examen histologique des gonades.

ВОЗДЕЙСТВИЕ РАДИАЦИИ НА ПОЛОВЫЕ КЛЕТКИ НАСЕКОМЫХ: ДОМИНАНТНЫЕ ЛЕТАЛИ, ИНАКТИВАЦИЯ ГАМЕТ И УМЕРЩВЛЕНИЕ ПОЛОВЫХ КЛЕТОК. Излучения и химические мутагенные вещества умерщвляют клетки различными путями: одним из видов образования доминантных леталей, непосредственной инактивацией функции, как, например, в отношении спермы, и генетически неопределимыми видами смерти, которые могут быть связаны или не связаны с доминантной летальностью. Химические мутагены производят, по-видимому, необычайное усиление оплодотворяющей способности спермы.

Различные стадии оогенеза и сперматогенеза реагируют с неодинаковой чувствительностью на облучение, и разница в чувствительности отдельных клеток на некоторых стадиях развития достигает 50 раз.

Там, где представляется возможным сравнивать двукрылых, перепончатокрылых и жесткокрылых насекомых, можно наблюдать замечательное единообразие реакции на облучение как по уровню радиочувствительности на определенной стадии, так и по зависимости степени реакции от дозы. Шелкопряд, Bombyx mori (отряд чешуекрылых), представляется похожим в большинстве отношений на представителей других отрядов по реакции половых клеток на радиацию, но резко отличается по типам индуцированных доминантных леталей. Виды с атипичным генетическим механизмом (например, леканоидная система Planococcus citri (отряд настоящих полужесткокрылых Coccidae)) представляют собою особые случаи, и их реакция на облучение значительно отличается от реакции насекомых других видов.

При истреблении популяций насекомых облучением самцов доминантная летальность представляется столь же перспективной для видов с однократным спариванием, как и для видов с многократным спариванием. Инактивацию спермы и умерщвление половых клеток можно считать примерами получения истинной стерильности, наиболее эффектной для видов, где спаривание происходит однократно. Для наиболее эффективного истребления должны выбираться такие дозы, которые ведут к получению максимальной доминантной летальности, производят минимальную инактивацию спермы и вызывают полное умерщвление половых клеток. Эти параметры легко поддаются определению посредством измерения жизнеспособности гамет, экспериментами с конкуренцией между облученными и необлученными популяциями, а также гистологическим исследованием гонад.

EFECTOS DE LAS RADIACIONES SOBRE LAS CÉLULAS GERMINALES DE LOS INSECTOS: ELEMENTOS LETALES DOMINANTES, INACTIVACIÓN DE LOS GAMETOS Y EXTERMINACIÓN DE LAS CÉLULAS GONÁDICAS. Las radiaciones y los productos químicos mutágenos pueden matar las células de modos diversos, a saber: mediante la inducción de uno o varios tipos de elementos letales dominantes, por inactivación directa de una función, como la producción de esperma, y por procedimientos genéticamente indefinibles de exterminación que pueden o no estar relacionados con la letalidad dominante intrínseca. Por otra parte, los productos químicos mutágenos parecen provocar un curioso aumento de la capacidad fertilizante de la esperma.

La radiosensibilidad varía según las diferentes etapas de la oogénesis y de la espermatogénesis, y las células pasan por fases en que dicha sensibilidad llega a ser 50 veces superior o inferior que en otras.

En la medida en que distintas especies de dípteros, himenópteros y coleópteros son comparables, puede observarse una sorprendente analogía en la radiosensibilidad, tanto según las etapas como según las dosis. La radiosensibilidad de las células germinales del gusano de seda Bombyx mori (Lepidópt.) parece similar en muchos aspectos a la de los insectos de otros órdenes, pero en cambio hay grandes diferencias en cuanto a los tipos de elementos letales dominantes que en él pueden inducirse. Las especies con mecanismos genéticos atípicos (por ejemplo, el sistema lecanoideo del Planococcus citri (Hemípt. ; Coccidae) constituyen casos especiales y su radiosensibilidad varía considerablemente en relación con la de otras especies.

Para la lucha contra los insectos por el método de irradiación de los machos, la creación de un elemento letal dominante ofrece en las especies donde las cópulas son múltiples las mismas ventajas que en las especies que copulan sólo una vez. La inactivación de la esperma y la exterminación de las células gonádicas pueden

considerarse como casos de auténtica esterilización que alcanzan su máxima eficacia sólo en las especies que copulan una vez. Para que la lucha contra los insectos sea eficaz hay que aplicar dosis que produzcan un factor letal dominante máximo, una inactivación mínima de la esperma y una exterminación completa de las células gonádicas. Estos parámetros se pueden determinar fácilmente con la medición de la viabilidad de los gametos, los experimentos con poblaciones irradiadas y no irradiadas y el examen histológico de las gonias.

INTRODUCTION

Dominant lethality, sperm inactivation, and gonia and gametocyte killing have been studied more in insects than in any other group of animals. These types of damage can be measured by counting the proportion of viable offspring from laid eggs, or by studying changes in sex ratio in parthenogenetic species. Cytological and genetic investigations can be used to augment simple dose-survival studies.

TYPES OF RADIATION DAMAGE

Through cytogenetic analyses, the different known types of dominant lethal, inactivation, and killing events during gametogenesis have been classified; these are summarized in Table I. Discussions of each of these types of radiation damage can be found in the references cited in the Table. There are two broad categories of lethal damage to gametes, induced dominant lethality and induced sterility. Dominant lethality is an effect on a sperm or egg that will kill the zygote some time during development. Sterility derives from the killing or inactivation of a gamete, or a cell that will become a gamete, so that it will no longer participate in formation of a zygote. As will be seen, the most common type of dominant-lethal effect is the Type I damage, that causing death of the embryo very early in development. This particular type is undoubtedly an expression of numerous kinds of damage, and these seem to vary in proportion from one mutagenic agent to the other. Probably cell killing itself, which causes depopulation of the gonial region of the testes, is mainly brought about by the same type of events that cause death early in development [1]. The Type IV damage, which results in immediate death, appears to be manifested only in certain kinds of cells. Cells such as lymphocytes, neuroblasts, young primary gametocytes, and late or secondary gonial cells respond by becoming pyknotic and dying relatively soon after irradiation without completing a mitotic division.

Recognizable sperm inactivation seems to occur in insects at doses beyond that dose needed to induce 99% dominant lethality. In the parthenogenetic Hymenoptera, sperm inactivation by radiation or exposure to chemical mutagens can be detected easily (Fig. 1). At low doses, hatchability is first reduced through induction of dominant lethality; thus fertilized eggs are killed. As the dose increases, hatchability returns to a higher level. The degree of return signifies the extent of sperm inactivation, because now the eggs are producing only haploid males. In species where fertilization is obligate, sperm inactivation is difficult to detect. The relative degree

TABLE I

TYPES OF DOMINANT LETHAL, CELL KILLING AND SPERM INACTIVATION EVENTS INDUCED BY RADIATION OR CHEMICAL MUTAGENS IN MATURE GAMETES AND DURING GAMETOGENESIS

Type	Effect	Probable basis	References
Ia	Death very early in development	Mitotic inhibition through chromosome bridge formation	[1]
Ib	Death very early in development	Mitotic inhibition induced by ultraviolet and reparable by photoreactivating light	[49]
Ic	Death very early in development	Mitotic inhibition reparable by presence of another nucleus (genetically non-transmissible damage, not dominant lethality sensu stricto)	[9,50-52]
Id	Death very early in development	Mitotic inhibition, non-reparable, but cause unknown	[51]
II	Late embryonic death before hatching in unfertilized eggs of Habrobracon and after hatching in fertilized eggs	Genetic death, through loss of chromosome parts needed for normal development	[21,50]
III	Late embryonic death before hatching in both unfertilized and fertilized eggs of Habrobracon	Genetic death, through loss of chromosomes or drastic loss of chromosome parts	[21,50]
IVa	Causes sterile period. Cells die before going through division. Death at G_2 to early prophase.	Unknown	[50,53,54]
IVb	Causes sterile period. Cells die just after going through division. Death at anaphase or early telophase.	Unknown	[54]
V	Dominant lethality induced in gonial cells	Possibly destruction of gene function or enhancement of normal aging processes	[9,12,50, 55]
VI	Sperm inactivation. Causes male sterility.	Unknown	[2-4,36-38, 56, 57]

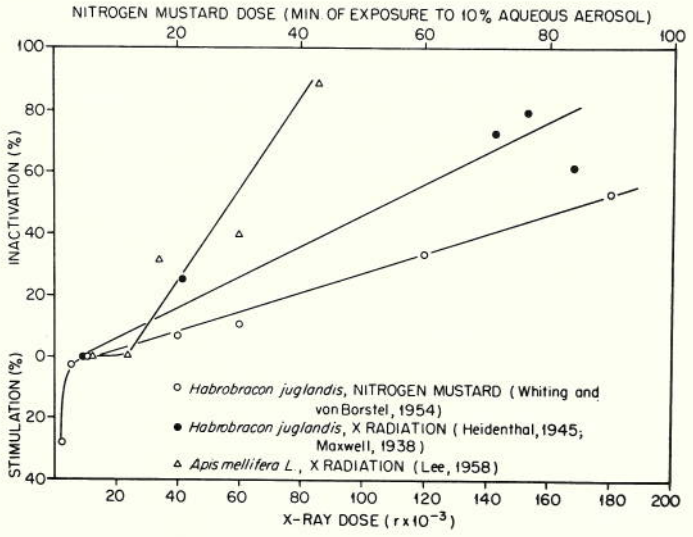

Fig. 1

Dose-inactivation curves of Hymenopteran sperm [36, 38, 56, 57]
Inactivation is measured as an increase in frequency of parthenogenetic embryo development to hatching, or maturity. At low doses, nitrogen mustard enhances the fertilizing capacity of the sperm.

of successful inseminations [2], and sperm competition experiments [3] indicate that sperm of the fruit fly, Drosophila melanogaster, are inactivated by radiation. These experiments should be repeated at higher doses. It has been shown in the white pine weevil, Pissodes strobi, [4] that virgin females mated with males irradiated at 10 000 and 20 000 r produced no viable offspring, whereas females previously mated with unirradiated males and again mated with males receiving the same doses produced more viable offspring at the higher dose, indicating inactivation of the irradiated sperm. Thus there was inefficient sperm competition by the sperm irradiated at 20 000 r.

The major difficulty in interpreting effects that can be ascribed to inactivation of sperm is that it is simulated by lack of sexual vigour of males, a response that could possibly be elicited by radiation. The problem is whether the competition is among males or among sperm. In parthenogenetic Hymenoptera the answer is clearly at the level of the sperm.

THE STIMULATING EFFECT OF ALKYLATING AGENTS ON SPERM

The same problem of competition among sperm or males exists also when alkylating agents, such as nitrogen mustard or some of the "chemosterilants" are used (Fig. 1). An interesting effect occurs at low doses of nitrogen mustard, "low" still being a dose that gives over 99% dominant lethality. This effect is one of apparent stimulation of the treated sperm to fertilize more eggs than normally would be fertilized. A similar effect ascribed to increased sexual vigour of males was found among house-flies,

Musca domestica (L.), treated with alkylating agents [5]. In any event, this effect, which enhances the fertilizing capacity of sperm in Habrobracon and the fertilizing capacity of sperm or mating competitiveness of males in house-flies, occurs beyond the 99% induced dominant lethal dose to sperm. For population control, this effect, properly employed, could be a powerful weapon.

DIFFERENTIAL SENSITIVITY OF MEIOTIC STAGES

This topic has been reviewed by WHITING [6] and has been the subject of a recent symposium [7, see especially 8]. The reasons for differential sensitivity are unknown, but action of repair mechanisms or presence of radical trapping agents at some stages and not at others seem to be among the most likely suggestions [8, 9].

Oogenesis

The differential sensitivity of the first meiotic metaphase and prophase of Habrobracon juglandis oocytes to two different alkylating agents and to X-radiation are shown in Fig. 2. It can be seen that in each instance metaphase-I oocytes are considerably more sensitive than are prophase-I oocytes. This shows that the phenomenon of differential sensitivity may well be of a general nature; i.e. the relative sensitivity of a given type of meiotic cell to certain mutagenic agents (particularly those other than purine and pyrimidine analogues) may be independent of the mutagen but dependent on the stage of the chromosome cycle [cf. 10].

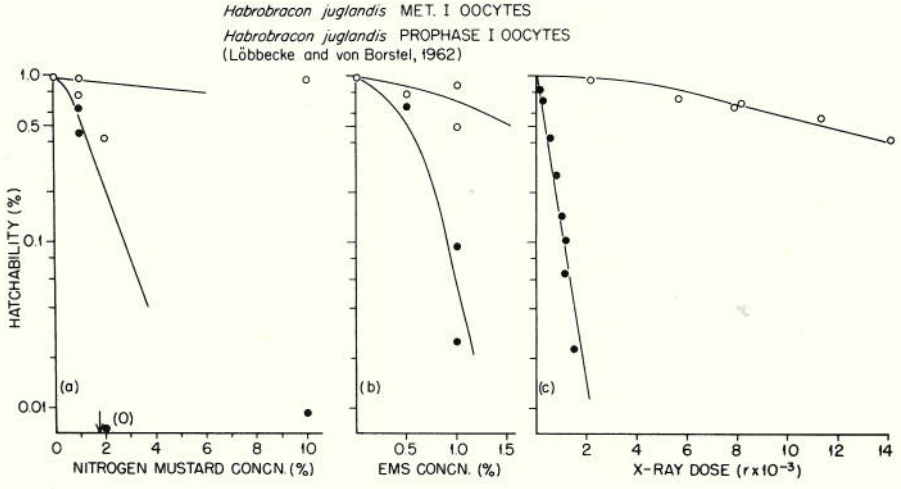

Fig. 2

Dose-hatchability curves for Habrobracon juglandis metaphase I and prophase I oocytes treated with (a) nitrogen mustard, (b) ethyl methanesulphonate (EMS), and (c) X-radiation [51].
●, metaphase I
O, prophase I

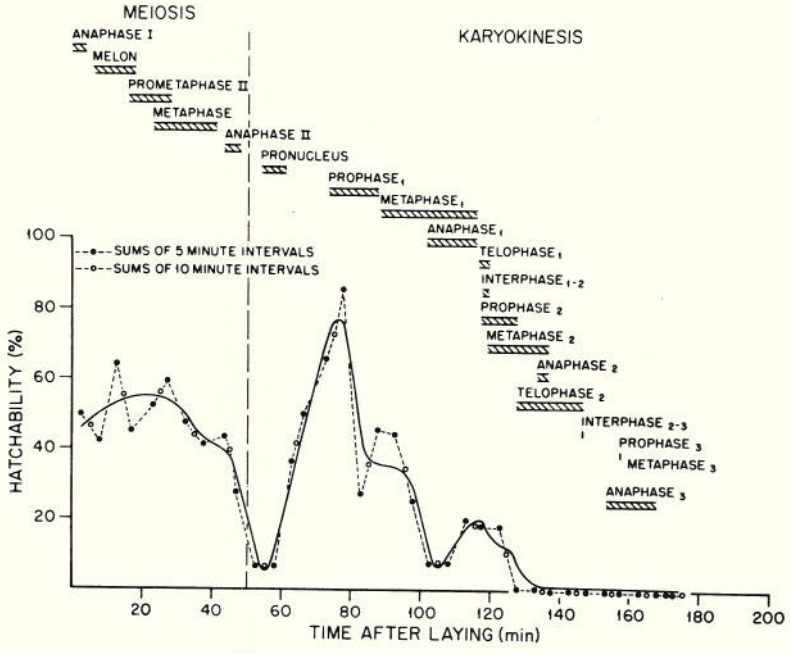

Fig. 3

Hatchability of H. juglandis embryos irradiated with 500 r at different times after laying [9]
Horizontal bars represent stages of meiosis and karyokinesis observed in embryos fixed at different times after laying.
---●---, sums of 5-min intervals
---O---, sums of 10-min intervals

The large magnitude of changes in radiation sensitivity with the meiotic stage in Habrobracon can be adjudged by comparing the resistance of the first meiotic prophase, shown in Fig. 2c, with the extreme sensitivity of the second meiotic anaphase and first mitotic telophase in Fig. 3. There is about a 60-fold difference in sensitivity between these stages but complete dose-action data are needed before an accurate quantitative difference in sensitivity can be assigned. Unmated females only were utilized in the Habrobracon experiments reported here, so the differences in sensitivity are a reflection not only of the different types of dominant lethality, but also of recessive lethality and genetically non-transmissible damage as well. Dominant lethality is known to account for at least 80% of the killing at all stages in Habrobracon.

That the sensitivity difference between metaphase I and prophase I occurs elsewhere in insects than in Habrobracon can be seen in Fig. 4. There is a striking similarity between the fruit fly and the screwworm in responses of metaphase I and prophase I oocytes [11, 12] to that of Habrobracon (Fig. 2c). In Drosophila literature and in the Figure these are referred to as stages 14 and 7 [13]. Cytological studies have not yet been thorough enough to determine if stage 14 is explicitly in the first meiotic metaphase, although it appears certain that stage-7 oocytes are equivalent to the prophase-I

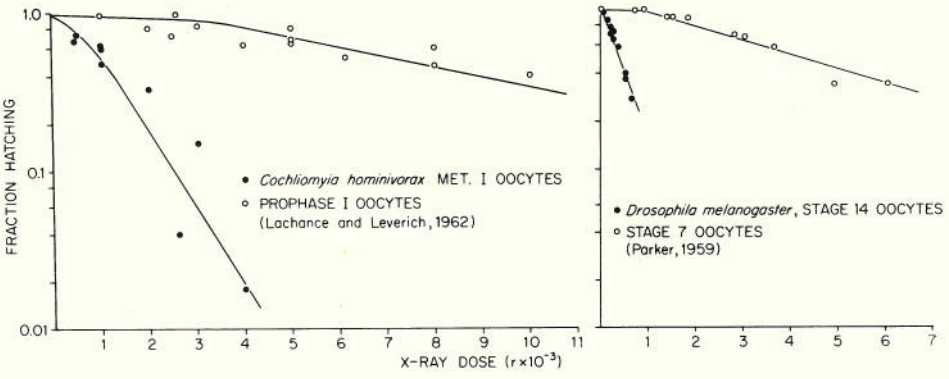

Fig. 4

Dose-hatchability curves for Dipteran oocytes irradiated in the first meiotic metaphase and prophase [Redrawn from [11, 12]]
Note similarity of stage sensitivity responses to H. juglandis (Fig. 2c).

oocytes listed for the other species. For stage 14, it is known that the nuclear membrane has disappeared, leaving a "karyosphere," a feulgen-positive body about the same diameter (2 μm) as the entire group of metaphase-I chromosomes (2.8 μm) in Habrobracon [27]. The striking similarity in the stage response to other species indicates indirectly that this stage may be the equivalent of metaphase I.

In Cochliomyia, data are also available for the dose-response of the first meiotic anaphase [12]. This stage has essentially the same sensitivity as the first meiotic metaphase, which is similar to the findings in Habrobracon (Fig. 3).

Apparently, similar oocyte stage responses also exist in the fungus gnat, Sciara coprophila [15], and the chalcid wasp, Mormoniella vitripennis [16, 17], but the data are limited and dose-survival curves cannot be plotted from the available data.

The dose-response of mature oocytes of the silkworm, Bombyx mori L. [18], presents certain difficulties for interpretation. The dominant lethal curve is convex upward to a marked degree (Fig. 5). Also the dose-hatchability data indicate, over the first part of the curve at least, that Bombyx metaphase I oocytes are more resistant than metaphase I oocytes of other insect species. One possible reason for this may be that the contracted chromosomes are not yet in a completely defined metaphase stage, since even though the chromosomes are discrete, the spindle has not yet formed [19, Figs. 133, 134]. A more likely reason for the different stage reponse lies in the observation of TAZIMA [20] that most dominant lethals induced in Bombyx cause death late in embryonic development. This is quite the reverse of that found in other insect species, and may set the Lepidoptera apart from other insect orders. A comparative radiobiological survey of the time of action of induced dominant lethals is needed.

Fig. 6 shows that the preponderant frequency of dominant lethality induced in oocytes of two different wasps, a braconid and an ichneumonid, results in death early in development [21]. The upper curves in each case represent 1.0 minus the proportion dying during early development. In

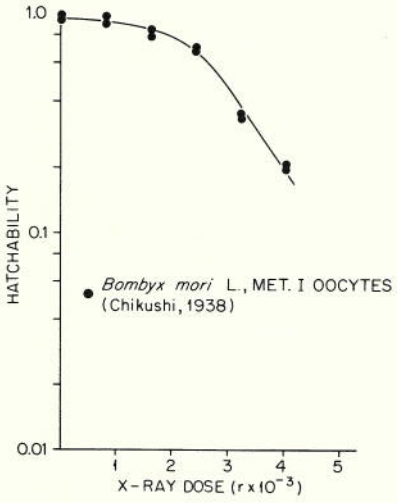

Fig. 5

Dose-hatchability curves for silkworm oocytes irradiated in the first meiotic metaphase [18]

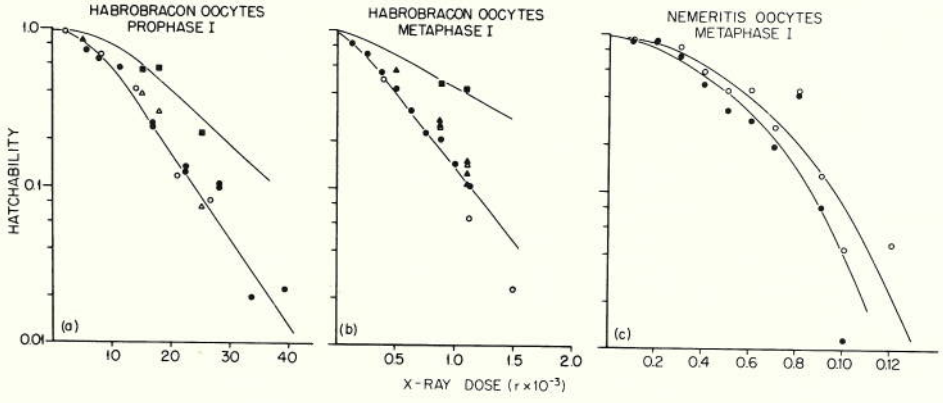

Fig. 6

Dose-hatchability curves for Hymenopteran oocytes
The upper curve in each case represents the fraction of induced dominant lethals that act early in development.
Hatchability data for (a) and (b) are from refs: [22] ●; [23] O; [24] ▲; [21] Δ
■ 1.0 minus the proportion dying before blastoderm formation [21]
Hatchability data for (c) are from:
● Speicher and Speicher, unpublished data
O 1.0 minus the proportion dying before blastoderm formation

Habrobracon irradiated at metaphase I, about half of the induced dominant lethality is expressed late in development. This group is composed of a mixture of dominant and recessive lethals (since the hatchability curve is obtained from irradiated virgins) [21-24], whereas the early death group contains all dominant lethals. Embryo recessive lethal mutations act only

after blastula formation and the early death group never reach the blastula stage. In Nemeritis, all deaths are from induced dominant lethality and almost all cause death early in development. Nemeritis canescens is arrhenotokous, virgin females producing only virgin female offspring.

Spermatogenesis

Many different facets of stage response during spermatogenesis have been examined in detail in Drosophila melanogaster [7]. Many of these are summarized in Fig. 7. All of the induced events have peaks that occur during spermatogenesis (as shown by successive matings of males on days following X-irradiation). Of interest to us is that dominant lethality is the broadest peak, comprising all the other types of analysable damage. This also indicates the possible heterogeneity of origin of induced dominant lethality, coming from a variety of phenomena associated with chromosomal breakage events as listed in Table I. This peak covers not only the spermatocyte divisions but the spermatid stage as well.

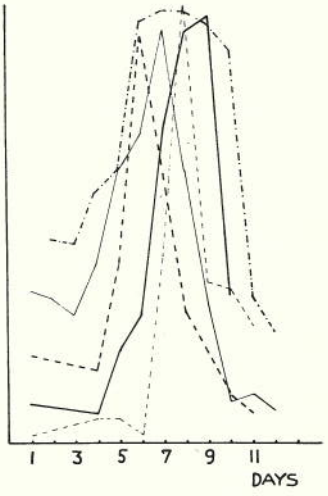

Fig. 7

Sensitivity patterns for different types of radiation effects at different stages of spermatogenesis in Drosophila melanogaster [29]

— · — · — Dominant lethals [25]
——— $\hat{X}XO$ females [26]
——— Recessive lethals [27]
- - - - - - Translocations [26]
------ Non-disjunction females [28]

Stage sensitivity during spermatogenesis for dominant lethality has also been observed in Tribolium (Fig. 8) by McDONALD [30]. He compares the dominant lethal peaks in Tribolium confusum at two different doses with the dominant-lethal peak observed in Drosophila virilis [31]. It is not known whether the same stages are the most sensitive, but the peak is large enough, as in Fig. 7, to encompass a considerable portion of spermatogenesis.

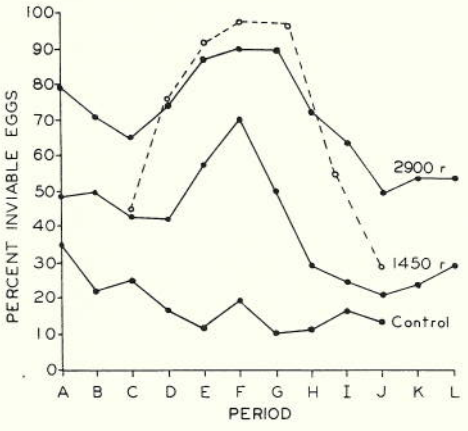

Fig. 8

A comparison of the radiation damage in the male germ cells of Tribolium confusum (solid lines) and Drosophila virilis (broken line) [30]. (The Drosophila data were taken from [31])

When males are irradiated, after the cells going through spermatogenesis are exhausted, a sterile period sets in. The sterile period indicates that early spermatocytes and secondary spermatogonia have been killed, as seen in Table II [from 44]. At lower doses, the spermatogonial region is not excessively depopulated and spermatogenesis is restored with consequent full fertility of the males. At higher doses (about 10 000 r in D. melanogaster), all cells in the gonial region are destroyed, and the male becomes completely sterile after the sperm supply is exhausted.

Sterile periods during spermatogenesis have also been observed in Bombyx by SADO [35]. Here the slow and fairly well synchronized development of gametic cells makes possible a separation of stages close to one another. He notes the good agreement in time of occurrence of cell killing during gametogenesis among species of Diptera, Lepidoptera, and Orthoptera.

Sperm

Induced dominant lethality has been investigated more in sperm than in any other cell type. Certainly sperm represent an extremely uniform population of cells that can be easily assayed.

In species of Diptera and Hymenoptera [21, 36] that have been studied by hatchability techniques there is a striking similarity of sensitivity to the X-radiation (Fig. 9). Here also it can be seen that the type of dominant lethality most often induced is that which results in death of the embryo early in development.

In some Hymenopteran species where it is difficult to record hatchability (such as those species where eggs are laid inside the host), accurate dose-survival curves can be formulated by observing the shift in sex ratio among progeny of the irradiated wasps. Curves plotted from data collected on two different chalcid wasps [37, 37a] and on Habrobracon [38] are shown in Fig. 10.

TABLE II

HISTOLOGICAL CONDITION OF DROSOPHILA TESTIS AFTER ADMINISTRATION OF 4000 r OF X-RAYS TO ADULT MALES*

Days after irradiation	Gonial region	Primary spermatocytes	Spermiogenic cysts
2¼	+	++	+++
3	++	+	+++
4	+++	0	+++
5	+++	++	+
6	+++	+++	0
7	+++	++++	0
8	+++	++++	0
9	+++	+++	0
10	+++	+++	+
11	+++	+++	+
12	+++	+++	+
13	+++	+++	++
14	+++	+++	++
15	+++	+++	+++

* The normal condition of a testis is designated by +++ [From 32].

Most dominant lethality is expressed in the embryo; this accounts for the similarity of slopes in Figs. 9 and 10 even though different parameters – on different species – are measured.

The silkworm Bombyx exhibits a dose-response for sperm (Fig. 11) that is strongly convex upward [39, 40]. This resembles the metaphase-I oocytes with respect to shape of the curve and also with repect to a general increase in resistance when compared with species in other insect orders. Again, few of the dominant lethals are expressed early in development [20].

A study of induced "sterility" has been performed on the codling moth, Carpocapsa pomonella L. [33]. Although the data as presented are difficult to assess, one gains the impression that the codling moth is like Bombyx in that the sperm are comparatively resistant to ionizing radiation. Similarly, the sperm of the European corn borer, Ostrinia nubilalis Hbn., are extremely resistant to radiation compared to wasps and flies [34]. Except possibly for the lack of early deaths in embryos carrying a dominant-lethal event, the basis of the sensitivity difference is not known. However, among insects, it appears that the Lepidoptera can be set apart from the other orders.

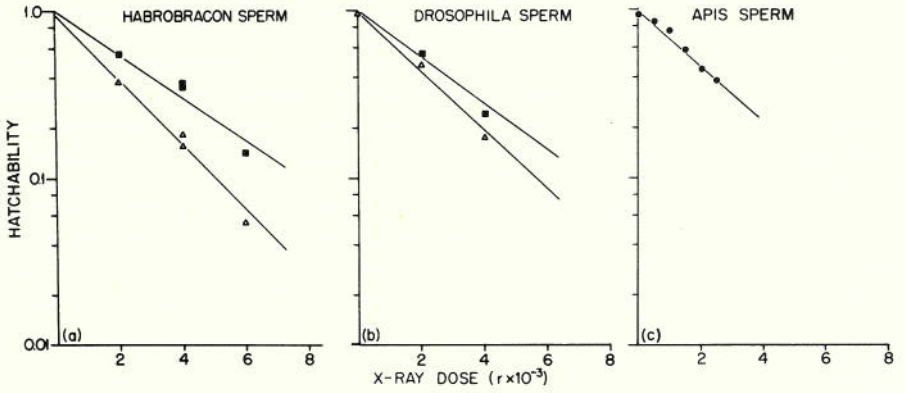

Fig. 9

Dose-hatchability curves for irradiated sperm of the wasp, Habrobracon; the fruit fly, Drosophila melanogaster; and the honeybee, Apis
The upper curve in two of the figures represents the fraction of induced dominant lethals that act early in development
Hatchability data for (a) and (b) are from [21] Δ, for (c) from [36] ●
■ 1.0 minus the proportion dying before blastoderm formation

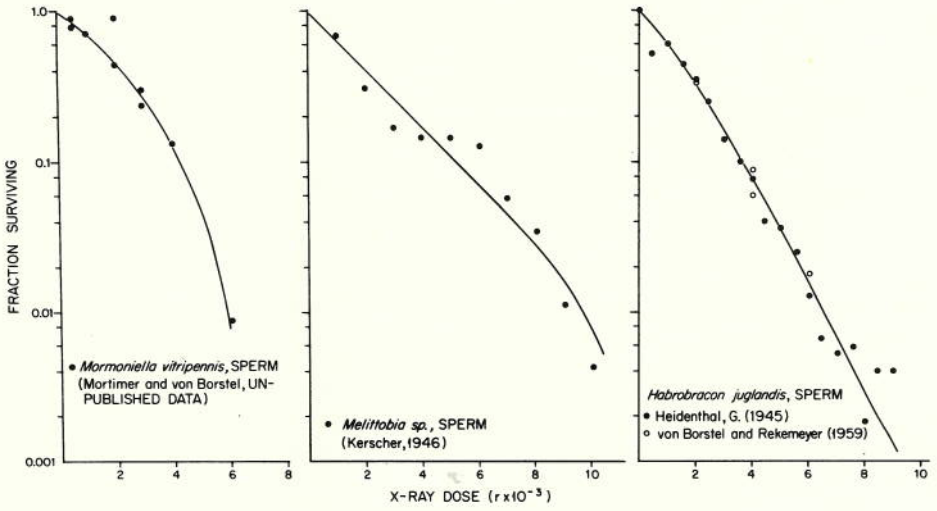

Fig. 10

Dose-survival curves for irradiated sperm of two chalcid wasps and one braconid wasp, computed from adult survival data where a sex-ratio shift was the criterion of induced dominant lethality [37, 37a, 38]

A considerable number of radiation experiments have been done on the Coleopteran Tribolium [30, 41-43]. There seems to be much variation in dose-response relations as measured by the different investigators for the two different species. It is unfortunate that the primary data are published only in one of the papers [34], so that inherent differences in methodology

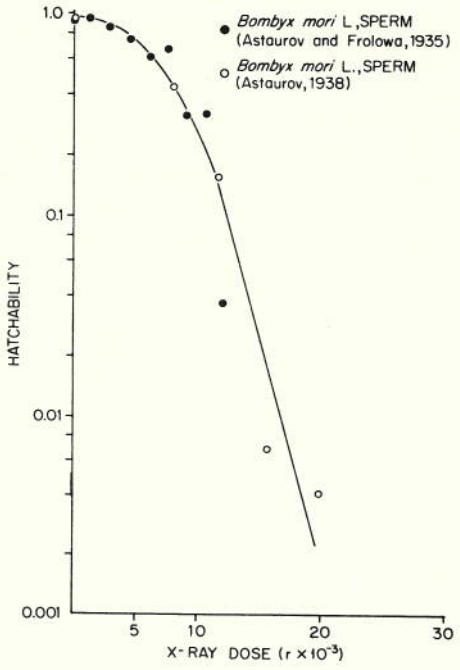

Fig. 11

Dose-hatchability curve for irradiated sperm of the silkworm, Bombyx [39, 40]

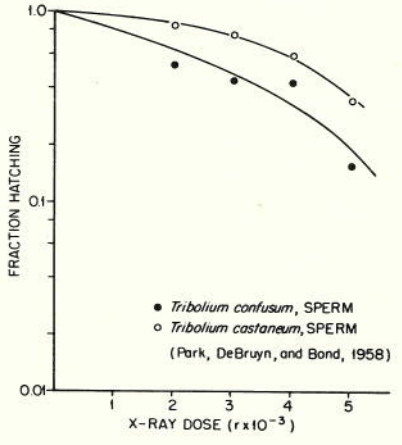

Fig. 12

Dose-hatchability curves for irradiated sperm of two different species of Tribolium [41]

and Tribolium strains cannot be properly gauged. The data in Fig. 12 indicate that a difference between two species of Tribolium may exist. The dose-action data do not differ markedly from that of Drosophila and the wasps, but the shape of the curve seems to differ somewhat.

An intensive investigation of the cytogenetics of Homoptera has been undertaken by Brown and his collaborators at the University of California. In a number of families in this order, one set of chromosomes in the male embryos becomes heterochromatic and is excluded from the genetic continuum during spermatogenesis. These chromosomes have been shown to derive from the male parent [44]. The results of irradiation of the sperm of the coccid Planococcus citri are shown in Fig. 13. Only the embryos destined to become females are killed at relatively low doses. At higher doses, females survive, and these derive both chromosome sets entirely from female origin. All embryos destined to become males survive up to doses of about 30 000 r. From here on there is a drastic reduction of this class. This indicates that even though the paternal set of chromosomes becomes heterochromatic in the male embryo, the sperm does contribute essential functional material for proper development [cf. 45] that is damaged only by very high doses of radiation.

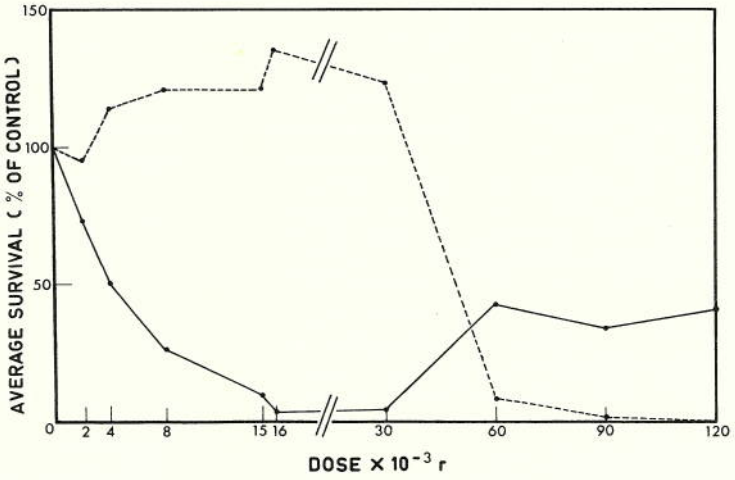

Fig. 13

Dose-survival curves for sperm of Planococcus citri indicating the average per-cent survival to maturity of embryos of both sexes [44]
—— ♀♀ ---- ♂♂

This lecanoid genetic system that Brown and his colleagues have investigated is restricted to certain families of Hemiptera. Other groups could be expected to respond to radiation in a manner similar to the more conventional responses found in Diptera and Hymenoptera. Variant genetic systems are found throughout the different orders of insects. Some of them could be used to augment population control by the irradiation-of-male method and others, like the lecanoid system, would offer special problems that would require special analysis.

DOMINANT LETHALITY AND STERILITY IN POPULATION CONTROL

The evidence indicates that there is a good deal of similarity of response to radiation from species to species, and even from order to order (Diptera,

Hymenoptera and Coleoptera) among insects, except for Lepidoptera. There is evidence that species in the Lepidoptera may be more resistant to radiation simply because the most frequent type of dominant lethality induced in other species is not induced here. A special genetic system in the Hemiptera presents special problems, and other variant genetic systems are known to exist in other groups of insects that could also vary responses to radiation [cf. 46].

It is known that induced dominant lethality causes competition to be at the level of the sperm rather that at the level of the male. Therefore the irradiation-of-male (lethal male) method for population control is equally effective whether the female mates once or many times [47, 48]. The essential data needed for any species for control purposes are: (a) the dose needed to achieve 99% or more dominant lethality in sperm; (b) the dose where sperm inactivation or reduced male vigour begins; and (c) the dose required to kill all the cells in the gonial region of the testes.

The rate of dominant lethality induced in sperm can be determined by hatchability measurements, and by extrapolation of the data on a semi-logarithmic plot to the 1% survival level. Sperm inactivation can be measured by simple multiple-mating experiments using irradiated and non-irradiated males. Killing of cells in the gonial region can be determined by histological examination of the gonial region of the testes a few days after irradiation to see if all the gonial cells are dead [32]. These same criteria and tests of male lethality are needed when alkylating agents are used instead of radiation.

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DISCUSSION

C. KRIMBAS: Dr. von Borstel, could you comment on the production of dominant lethals in Homoptera in view of the existence of a diffuse centromere as Schrader has shown?

R.C. von BORSTEL: I suspect that diffuse centromeres would reduce sensitivity where dominant lethality is the criterion. This may in fact explain the increased resistance in Lepidoptera. Do they not have diffuse centromeres?

C. KRIMBAS: The existence of a diffuse centromere has been claimed but not demonstrated in Lepidoptera. It would explain their resistance, and perhaps that of some Coleoptera.

R.C. von BORSTEL: Yes, in general it would tend to reduce the sensitivity, but the precise effect would be different in each case. No experi-

ments have, in point of fact, been done on dominant lethality in species which should be looked into. There have been radiation experiments, of course. What is done is to break up the chromosomes, but then they go to the poles, and no one has studied dominant lethality.

G.T. SCARASCIA: I should like to know if other alkylating agents, such as diethyl sulphate, which are of very little effect in breaking up chromosomes, at any rate in plants, have been successfully used for the induction of dominant lethals. If so, the dominant lethality cannot be strictly correlated with the chromosome aberrations, can it?

R.C. von BORSTEL: Yes, ethyl methanesulphonate, for instance, causes dominant lethality, and again the metaphase and prophase are differentially sensitive. As you say, this indicates that not all dominant lethality need necessarily be a result of chromosome breakage. Apparently it is effected through a slow-down of mitosis, but the actual mechanism behind dominant lethals induced by ethylmethene sulphonate is not known at all.

P. NARDON: Much has been said about dominant lethality, but it should not be thought that the effect of irradiation is exclusively mutagenic. It is very tempting when irradiating sperm to accept dominant-lethal mutations, but when irradiating oocytes, the possible effect of cytoplasm should not be forgotten. Various experiments have shown this. The work of Nakao on the silkworm, in particular, shows the lethality of irradiated cytoplasm to chromosomes, and the work of Duryee shows that isolated nuclei are much resistant.

R.C. von BORSTEL: I am aware of the work of both these investigators. Their results are very interesting, but difficult to interpret on the basis of nuclear injury. It should not be forgotten that heavily irradiated cytoplasm of eggs of Habrobracon juglandis Ashmead and Bombyx mori L. can still support embryonic development from unirradiated sperm with no effects visible upon the offspring. It has been shown that one alpha particle passing through the nucleus is enough to kill an embryo, but 20 000 000 must strike the cytoplasm before the embryo will die. We were able to show that this is not through any influence back on the nucleus because the types of killing are different. The same kind of experiment has also been done by Astaurov et al. in Russia. He has shown that in the silkworm at doses of approximately 200 000 r, or even higher, you can sometimes get double fertilization in the egg; the nuclei will join and they will develop normally. This effect has also been noticed by Japanese workers and remains unexplained – though I do not think it is of importance here.

K.K. NAIR: You have suggested the plotting of one's data on semi-log paper for calculation of the target number. If one were scoring lethality in an adult insect, would it be possible to calculate the target number in that way?

R.C. von BORSTEL: The question is how many parameters can be computed from two-dimensional data. Some progress has been made on the formal kinetics but the general usefulness is probably limited. One needs to be able to compute: (1) the sensitivity of the target; (2) the total number of targets in the sensitive tissue, for instance; and (3) the number of targets that, when inactivated, will cause the death of the organism. It appears now that all these parameters can be derived from a simple curve but the

problems are enormous. We can go into that in private if you like because all the problems have not yet been solved.

E. HORBER: For our irradiation work on cockchafers we have to decide which type of mobile equipment to acquire, in order to combine the best performance, from the radiobiological point of view, with technical and economical efficiency. Co^{60} or Cs^{137} units have the disadvantage of being rather too bulky and heavy to be used as mobile units, and radiation cannot be stopped when the unit is not in use. Safety measures have to be taken whether the unit is operated or not. These disadvantages are not encountered with X-ray units. Besides the standard X-ray units available in hospitals, new types with a beryllium window are coming into use. They emit a spectrum containing soft radiation. This, being more efficient as expressed in RBE values, is at the same time absorbed to a higher degree in the irradiated material. Therefore one would have to irradiate smaller quantities or thinner layers. The question I am asking the speaker as well as the audience is, what is the best type of mobile unit for our type of field work.

R.C. von BORSTEL: I refer that one to the audience.

B.D. BAINES: In answer to Dr. Horber's question, I would like to suggest that an isotopic source is absolutely dependable, and that an irradiation unit employing such a source can be made sufficiently rugged to operate effectively under field conditions. Of the common sources, Co^{60} and Cs^{137}, the latter is to be preferred as it does in fact permit the construction of the smaller, lighter units which are essential for mobile operation. It would seem probable that such a unit with an irradiation volume of one litre could be constructed with a weight of about one ton, suitable for transport in a van or trailer. I am sure that such a unit, which can never be "switched off", is better than a unit which may fail to operate when switched on.

G.T. SCARASCIA: I would like to ask whether, for induction of the gross chromosomal aberrations with which dominant lethality is generally correlated, densely ionizing radiations such as neutrons have been employed.

R.C. von BORSTEL: Yes, mostly with Drosophila. All these things depend upon the three purposes we are aiming at. One is to kill the gonia, another is to kill the sperm and the third is to inactivate the sperm. Gamma radiation seems to be ideal for pest control research in this connection. As far as I know, there have been no experiments at all on what are the different frequencies of the gonial killing and the inactivation cross-sections obtained by these means, although much is known about dominant-lethal cross-sections obtained with neutrons and other kinds of radiation.

THE EFFECTS OF GAMMA RADIATION ON THE OVARIES OF DACUS OLEAE GMEL.

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Abstract — Résumé — Аннотация — Resumen

THE EFFECT OF GAMMA RADIATIONS ON THE OVARIES OF DACUS OLEAE GMEL. The authors have studied by cytological and ultramicroscopic techniques the ovaries of unfertilized adult females of Dacus oleae irradiated in the middle period of the pupal stage with several doses (2-30 kr) of gamma rays. In all cases the treatment inhibits the normal development of the ovary. Nurse cells and egg cells are very small and few in number and show abnormal structure and ultrastructure, particularly as regards the cytoplasmic organelles.

EFFET DES RAYONS GAMMA SUR LES OVAIRES DE LA DACUS OLEAE GMEL. Les auteurs ont fait appel aux techniques cytologiques et ultramicroscopiques pour étudier les ovaires de femelles adultes de Dacus oleae non fécondées, qui avaient été irradiées au milieu du stade pupaire par plusieurs doses (2-30 kr) de rayons gamma. Dans tous les cas, l'irradiation a empêché un développement normal de l'ovaire. Les cellules nourricières et les ovules étaient très petits et rares, avec une structure anormale, surtout pour les organites du cytoplasme.

ВОЗДЕЙСТВИЕ ГАММА-ИЗЛУЧЕНИЯ НА ЯИЧНИКИ DACUS OLEAE. Авторы изучили с помощью цитологических и ультрамикроскопических методов яичники неоплодотворенных женских особей Dacus oleae, облученных в середине фазы окукливания различными дозами (от 2000 до 30 000 р) гамма-лучей. В каждом случае это тормозило нормальное развитие яичников. Питающие и яйцевые клетки были очень маленькими, количество их незначительно, структура и ультраструктура ненормальные, прежде всего в отношении

EFECTO DE LAS RADIACIONES GAMMA SOBRE LOS OVARIOS DE LA DACUS OLEAE GMEL. Los autores de la memoria han estudiado con técnicas citológicas y ultramicroscópicas los ovarios de hembras adultas, no fertilizadas, de la Dacus oleae irradiadas durante el período medio de la fase ninfal con varias dosis de rayos gamma (de 2 a 30 kr). El tratamiento inhibe el desarrollo normal del ovario. Los oocitos, en todas sus fases, son muy pequeños, aparecen en escaso número y muestran una estructura y ultraestructura anormales, sobre todo por lo que respecta a los orgánulos citoplásmicos.

INTRODUCTION

The modifications that ionizing radiations induce on the ovary have been observed ever since the beginning of radiobiological research: studies in this field were begun simultaneously in 1905 by HALBERSTAEDTER [1] in Germany and by BERGONIÉ et al. [2] in France.

The investigations were soon extended even to man, and functional studies were added to morphologic ones [3] with the observation of the phenomenon of temporary or permanent sterility.

The many researchers who have dealt with this subject [4, 5, 6, 7] have reached practically the same conclusions on the damage caused on the different ovarian structures of mammals by various types of electromagnetic

and particle radiation from both external and internal sources [6, 8, 9, 10, 11, 12].

In a recent article [13], ERRERA, FORSSBERG, RUGH et al. have made a full and up-to-date synthesis of the morphological and functional alterations of the ovary in the most common laboratory mammals examined by optical microscope. In animals irradiated with a LD50/30 whole-body dose, a rapid disappearance of the egg-cells of the primary follicle, associated with the destruction of the follicular cells of the more mature follicles, is observed. The germinal elements soon disappear. The number of luteal bodies slowly decreases, since they are no longer produced by the follicular cells which have now been destroyed. The interstitial tissue tends towards slow atrophy and in about two months' time the ovary consists of fibrous tissue containing cystic formations.

The egg-cell shows a different sensitivity according to its degree of development; the young or primary follicle is more sensitive than the secondary follicle.

The converse behaviour is exhibited by the cells of the granulosa: in the primary follicle stage they are more resistant than the oocytes, whose residues they eliminate when the oocyte is destroyed, whereas they are much more sensitive than the egg-cell in the mature follicle, and they constitute, in this phase, the most radio-vulnerable units in the ovary.

If the follicular cells are irradiated at the stage when they constitute an element of the luteal body, they are relatively resistant, at least so far as morphological examination by optical microscope reveals, and this fact is true for all mammals [14].

The covering epithelium is also fairly insensitive and may show a flattening of the cellular elements without signs of degeneration. In the interstitial tissue, radiation causes a slow process of atrophy to set in.

The morphological damage caused by radiation in the more sensitive cellular elements is revealed by examination under the optical microscope as consisting in alteration of the nucleus, which shows a thickening of chromatin reaching the extreme stages of pyknosis and karyolysis, and of the cytoplasm, in which vacuolization effects occur.

In insects too, the post-irradiation alterations of the gonads have been accurately studied and fully documented under the optical microscope by a number of authors [15, 16, 17, 18], particularly by ERDMAN [18].

Generally speaking, the doses necessary for the alteration and destruction of ovarian tissue are much higher in insects than in mammals, although they vary in a given animal according to the stage of development of the germinal elements.

The morphological alterations observed by Erdman in Habrobracon juglandis consist in atrophy of the ovarioles, which may be reduced to rudimentary appendages or "horns"; these are most frequently observed after irradiation at sterilizing doses (2500-4500 r) of the final stages of larval life.

Of the various segments of the ovariole, the most damaged is the germarium: its cellular elements appear to be constantly altered and are often reduced to fragments. The oocytes present various degrees of alteration, which range from a sometimes considerable shrinking of the whole cell, with

deformation and wrinkling of the nucleus, to the fragmentation of the cellular elements. Gross changes also appear in the nurse-cells.

In the pupal stage, after a certainly sterilizing dose of irradiation (3900-5700 r), which, in contrast to the situation for larvae, is lower than the lethal dose, one can observe deformation of the ovarioles, whose only well-preserved part seems to be the layer of monostratified epithelial cells that surrounds the ovarioles, whereas the other germinal elements (oogonia, oocytes and nurse-cells) are difficult to identify, being largely reduced to fragments.

Electron microscope study of the fine cellular structures, which has recently been added to the conventional histological examination, permits a more detailed analysis of the post-irradiation alterations of cellular organelles. However, we have not been able to trace that any research of this kind has been carried out on insects, whereas mammals have been the subject of many important works [20, 21].

The ultrastructural alterations observed in PARSONS' study on mice subjected to various doses of whole-body irradiation (7-200 r) of course varied in degree, according to the stage of development of the egg. These alterations mostly concern the nucleus, in which phenomena of kariolysis and pyknosis and nuclear alterations occur; they also concern the mitochondria, which decrease in number and size, and the RNA granules, which are reduced in numbers.

The present paper concerns the study of post-irradiation alterations in the ovary of Dacus oleae Gmel.; this insect has already been the subject of insect control tests using the sterile-male method [21], and of research on the effects of radiation on mesenteron. The object of this study is the evaluation of the lesions that various doses of certainly sterilizing radiation induce in the female germinal cells. We have employed the most modern methods of cytological investigation, such as the electron microscope and some histochemical reactions. Since research of this kind is completely absent in the literature, not only on irradiated insects but even on normal insects, we have had to begin our work by a study of the ultrastructure of the ovary in normal Dacus oleae females.

MATERIALS AND METHODS

The insects were irradiated in the pupal stage by the technique we have already described in earlier papers [22, 23]. We used a cobalt-60 teletherapy unit with F. D. 80 cm and r/m 30. For irradiation the pupae were placed side-by-side in a depression cut into the top of a block of paraffin-wax to a depth equal to the width of the pupae. The depression was covered with a layer of Plexiglass 5 mm thick. In this way we obtained a homogeneous irradiation of the insects and were able to keep the quantity of diffused radiation constant in the various tests.

We irradiated 8 lots of pupae from the Palermo area and administered in a single run, by separate groups, doses of 2, 4, 5, 8, 10, 12, 20 and 30 kr for times varying from 1 h 7 min. to 16 h 40 min. The irradiated pupae were then left to develop along with appropriate controls. When the adults

had emerged from the puparium, we selected, as in earlier studies, those that had been irradiated in the middle period of the pupal stage.

Some samples were fixed alive in Duboscq Brazil, embedded in paraffin, sliced in series and stained with Feulgen and Brachet reagents. Others were studied with the electron microscope. The fixation was carried out at + 4°C, with 1% osmium tetroxide buffered at pH 7.2 injected in vivo. The ovaries were removed by dissection, embedded in methacrylate and sliced with the Porter-Blum ultra-microtome.

The sections were then stained with 1.5% uranylacetate, covered with a thin protective coating of carbon and examined under a Hitachi HS-6 electron microscope.

RESULTS

(A) Normal structure of the ovary

The female of Dacus oleae, immediately after the emergence, usually shows well-developed ovaries containing mature eggs ready to be fertilized.

Each ovary is more or less conical in appearance, consists of 15 to 20 ovarioles converging into the calyx of the lateral oviducts, and generally speaking measures about 700 × 500 μm. Each ovariole is composed of a string (vitellarium) of 4–7 ovarian chambers that are quite easily identifiable and of a distal germarium containing the oogonia, the segmentation of which is less clear. The wall of each ovariole is formed by a single-layer follicular epithelium (Figs. 1 and 2), composed of small cuboidal cells. These cells are easily differentiated only in the vitellarium, where they have octoploid nuclei [24] that are rather small (about 4.5 μm in diameter), spheroid, full of positive Feulgen substance arranged in thin granular filaments, and contain only one nucleolus. The cytoplasm is rather sparse but rich in RNA, as is demonstrated by the pyroninophilia which is easily identifiable by Brachet's method and is homogeneously distributed around the nucleus.

Under the electron microscope (Fig. 9), these cells are seen to be extraordinarily rich in ergastoplasm (the cause of the pyroninophilia), in the shape of more or less twisted reservoirs and of free ribosomes.

The mitochondria, on the other hand, are small, sparse and poor in cristae; the multivesicular bodies and other organelles are few. The Golgi fields are constituted by swarms of tiny vesicles. The cell membranes often appear interrupted and show signs of frequent exchanges. In the nucleus, the chromatin is in the form of granules, and is homogeneously distributed; the karyotheca is provided with many pores with annuli and continually produces ergastoplasmic cysts by means of vesiculations of its lamina externa.

In the germarium (Fig. 3) the follicular cells do not seem separable from the oogonia, which will be described further on.

The follicular epithelium lies on a thin basal membrane (Figs. 9 and 13), which has been found under the electron microscope to be 2000-3000 Å thick and made up of very thin filaments (about 200 Å thick) of indefinite length. These filaments show along their length an alternation of dark and light stripes, every 600 Å or so, and are quite similar to those that

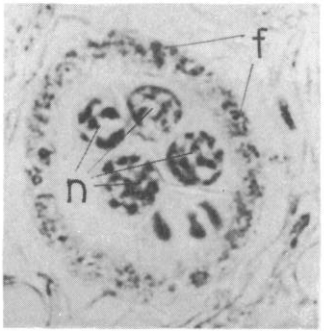

Fig. 1

Cross-section of ovariole of a normal Dacus oleae Gmel. female
(n), nuclei of the nurse-cells; (f) nuclei of the follicular cells. Feulgen's reagent, 560 diam.

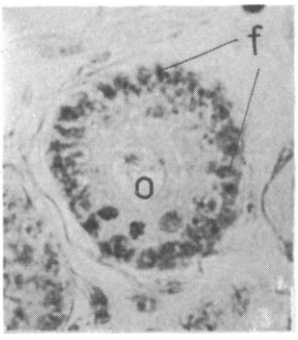

Fig. 2

Cross-section of the same ovariole, on another level
(f), follicular cells; (o), nucleus of oocyte. Feulgen's reagent, 560 diam.

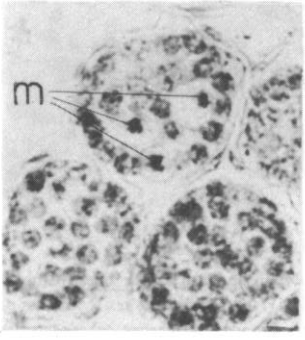

Fig. 3

Cross-sections of three ovarioles in a normal Dacus oleae Gmel. female, sectioned at the level of the germarium
Many of the oogonial nuclei are in mitosis (m). Feulgen's reagent, 560 diam.

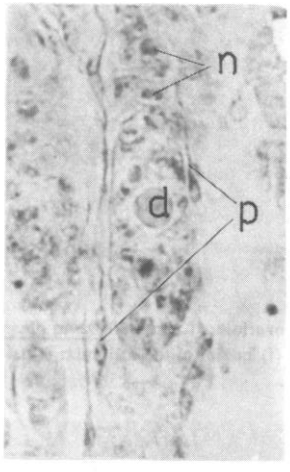

Fig. 4

Longitudinal section of ovariole of a Dacus oleae Gmel. female irradiated at the pupal stage with 2 kr. In the inner part of the peritoneal membrane (p) one can observe many undifferentiated oogonia (n) and a few cells in degeneration (d). Pyknoses are completely absent. Feulgen's reagent, 560 diam.

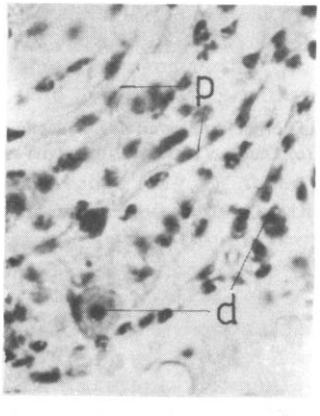

Fig. 5

Oblique section of a clump of ovarioles in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 12 kr

Abundant nuclei can be seen in the peritoneal membrane (p) and, in the inner part, a paucity of cells, of which a few are degenerating (d), can be observed. Feulgen's reagent, 560 diam.

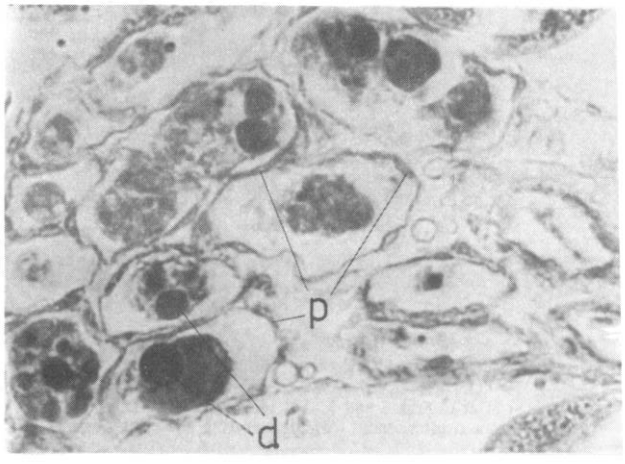

Fig. 6

Cross-section of a clump of ovarioles in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 20 kr.
In the inner part of the peritoneal membranes (p) which are intact, a small number of cells can be seen, all of them degenerating (d), with a pyknotic nucleus and highly pyroninophile cytoplasm. Brachet's reagent, 560 diam.

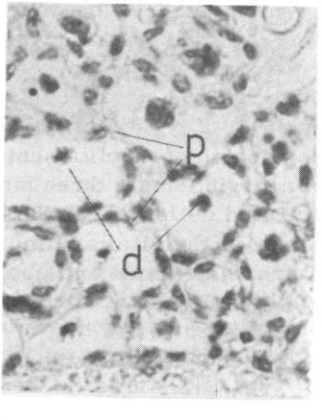

Fig. 7

Similar to Fig. 6, but with the nuclear structure more clearly brought out by Feulgen's reagent
In the inner part of the peritoneal membrane (p), very few nuclei, all degenerating (d), are visible.
Feulgen's reagent, 560 diam.

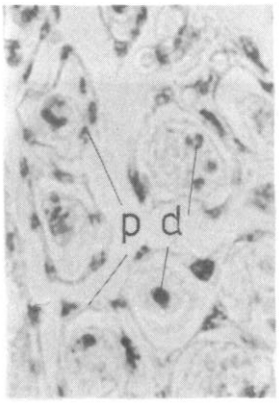

Fig. 8

Similar to Figs. 6 and 7, but after dose of 30 kr
(p), peritoneal membrane; (d) degenerating nuclei of oogonia.

BACCETTI [24] described in the nervous system of other Diptera. On the basis of our present knowledge of the connective tissue of insects, they may be assumed to be collagen.

Each ovariole is surrounded by a robust peritoneal membrane, nucleated and highly PAS-positive. The electron microscope shows it to consist of very broad, flattened cells (Figs. 9, 14 and 15) lying between two basal membranes which are homogeneously granulated and about 1000 Å thick.

These cells have small nuclei, with intact karyotheca having few pores, very abundant cytoplasm containing small ergastoplasmic formations, small mitochondria, well-marked Golgi fields and myofibrillae with well-marked myofilaments, clearly of the striated type. The cytoplasm is also furrowed by tracheoles and by minute tubules of sarcoplasmic reticulum: it shows, in other words, all the features of typical muscular tissue, with the special characteristic of a striking dearth of myofilaments.

Inside the ovariole of a virgin female three main types of cell are found: the oogonia, non-differentiated cells concentrated in the germarium, the oocytes, and the nurse-cells in the vitellarium.

The oogonia (Fig. 3) are very small and poor in cytoplasm, which is always weakly pyroninophile: they have spheroidal nuclei about 6 μm in diameter and frequently go into mitosis.

Under the electron microscope (Fig. 13) the oogonia with resting nucleus seem to be rather poor in organelles; one observes a few vesicular mitochondria with low internal cristae, spheroidal ergastoplasmic cysts rich in ribosomes, and some scattered Palade granules.

The Golgi apparatus is not evident; the cell boundaries are very tortuous; they almost always appear to be intact and show no signs of exchanges between adjacent cells. The nucleus seems to be filled with a coarse granular substance, very homogeneously diffused and rather thick, and contains only one rather small nucleolus. The karyotheca has few pores; its two osmiophilic layers are in many cases hollowed out to a considerable

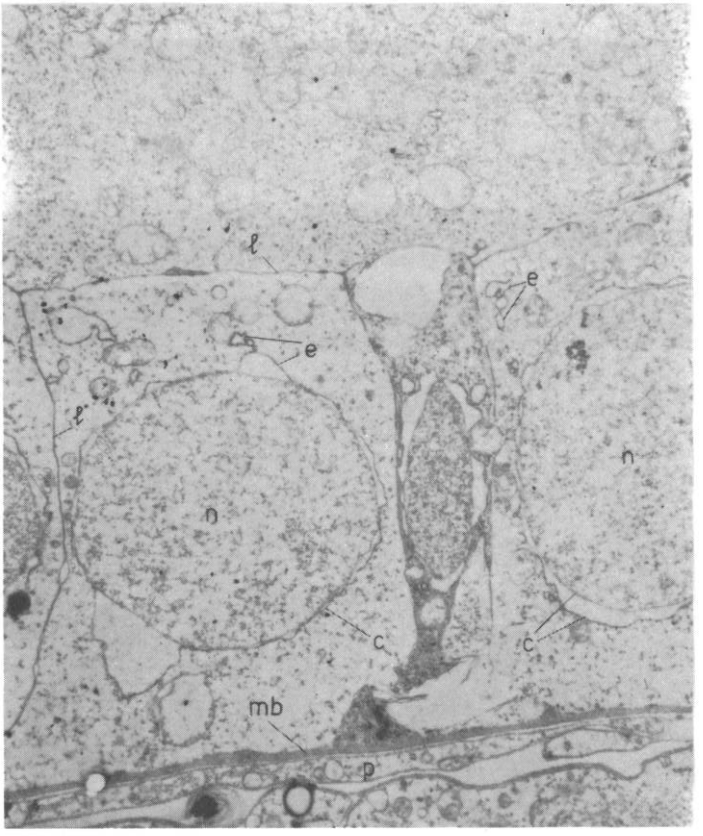

Fig. 9

Electron micrograph of the cortical zone of the ovariole in a normal adult Dacus oleae Gmel. female The section was made on the level of the vitellarium. In the upper part of the figure a portion on a nurse-cell can be seen. In the lower part, grouped together, there are some follicular cells, whose richness in ergastoplasm is evident. The ergastoplasm clearly originates in the outer layer of the karyotheca. (c), karyotheca; (l), cell membranes; (e), ergastoplasm; (m) mitochondria; (mb) basal membrane; (n) nuclei of the follicular cells, (p) peritoneal membrane.
Hitachi HS-6, 11 200 diam.

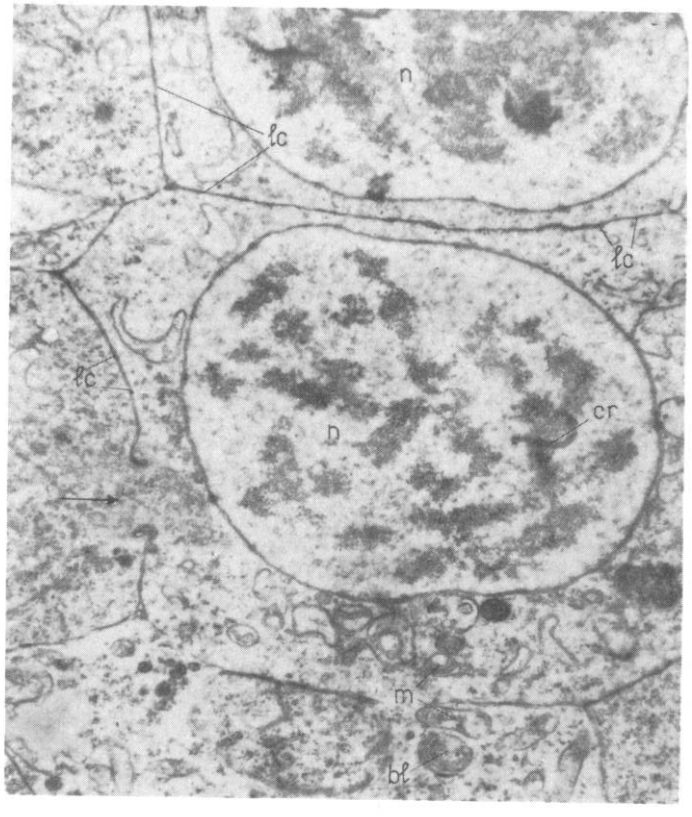

Fig. 10

Electron micrograph of some adjacent nurse-cells in the vitellarium of an ovariole in a normal adult Dacus oleae Gmel. female

Note the presence in the nuclei (n) of segments of polytene chromosomes (cr), and the richness of the cytoplasm in mitochondria (m) and lamellar bodies (bl). The cell membrane (lc) is very straight, but seems to be interrupted at the points indicated by an arrow, where an intense migration of substances can be seen. Hitachi HS-6, 11 200 diam.

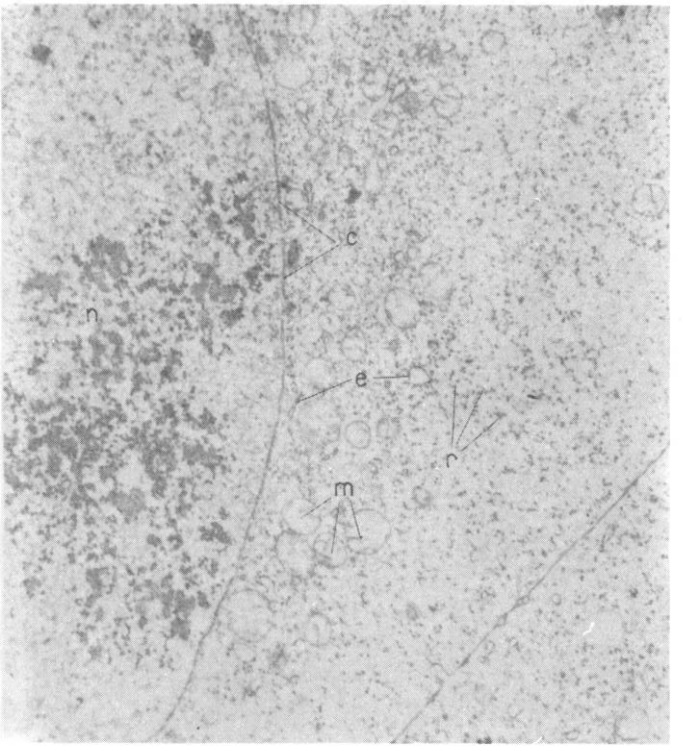

Fig. 11

Electron micrograph of a group of nurse-cells which have attained complete growth in the same subject as Fig. 10.

Note the large size of the nucleus (n) whose karyotheca (c) has no relation of continuity with the ergastoplasmic cysts (e) surrounding it. Besides these, many mitochondria (m) and free ribosomes (r) can be seen in the cytoplasm.

Hitachi HS-6, 11 200 diam.

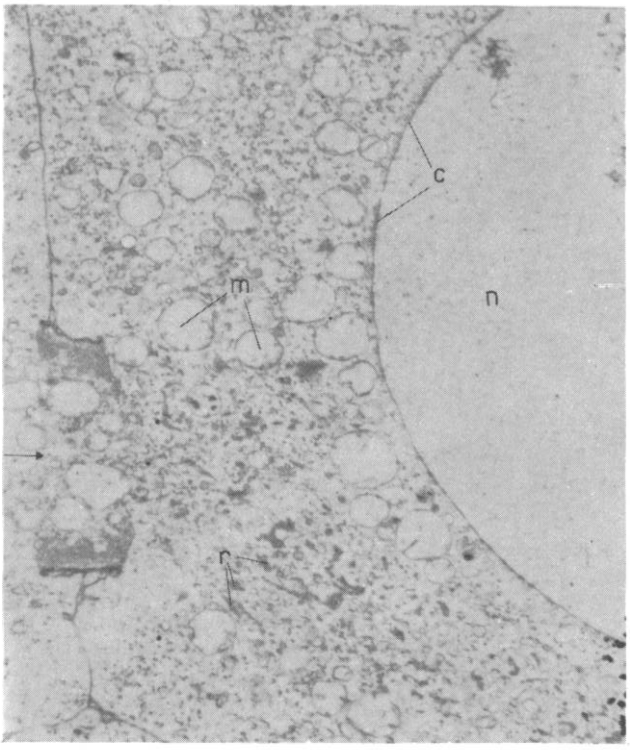

Fig. 12

Electron micrograph of a portion of an oocyte in the same subject as Fig. 10
Note the nucleus (n), very poor in chromatin and with intact karyotheca (c). In the cytoplasm there are many mitochondria (m) and free ribosomes (r), organelles from the neighbouring nurse-cell are passing through a wide opening (indicated by an arrow) in the cell membrane.
Hitachi HS-6, 11 200 diam.

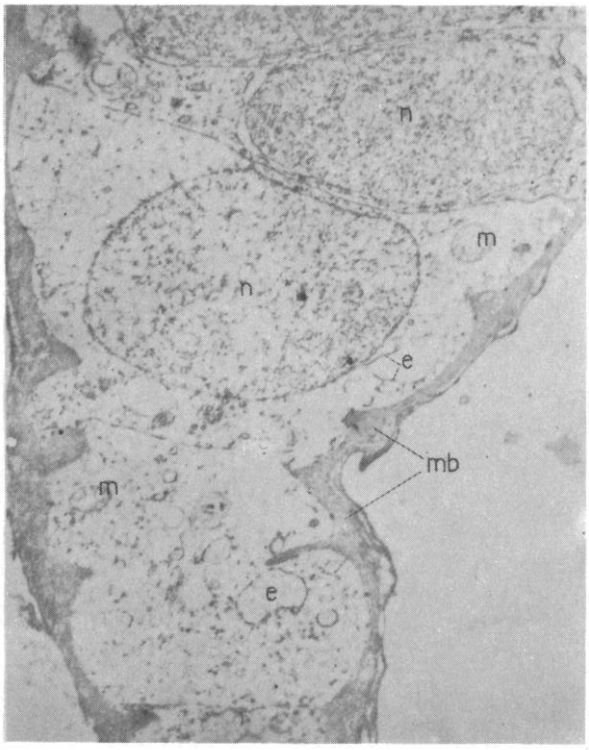

Fig. 13

Electron micrograph of a longitudinal section of the extremity of the germarium in an ovariole belonging to the subject of Fig. 10.

Note the thick basal membrane (mb) which surrounds the oogonia sideways. The oogonia are densely grouped, with large nuclei (n) and cytoplasm rather poor in organelles, limited to some mitochondria (m) and a small amount of ergastoplasm (e).

Hitachi HS-6, 11 200 diam.

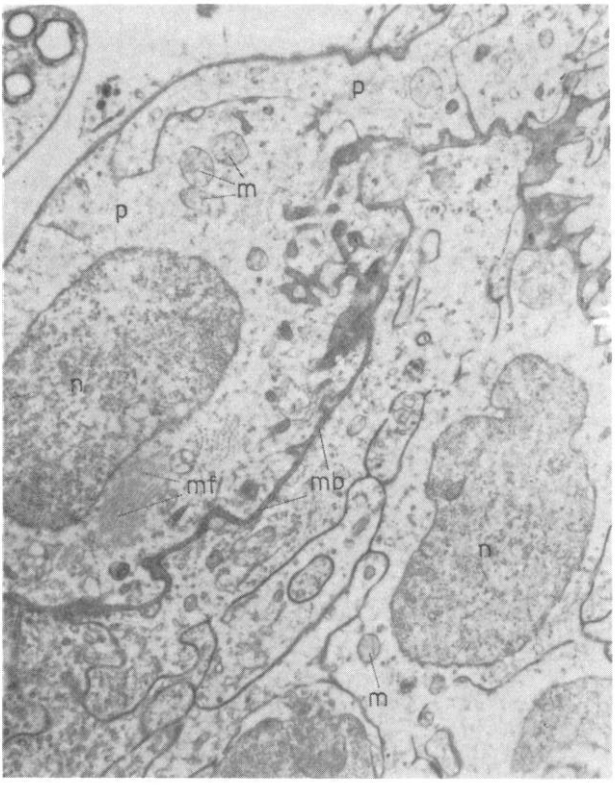

Fig. 14

Electron micrograph of the region corresponding to the vitellarium in an ovariole of adult Dacus oleae Gmel. female irradiated at the pupal stage with 5 kr

Note the perfect preservation of the peritoneal tunica (p) in which a nucleus (n), some mitochondria (m) and myofilaments (mf) can be seen. In the inner part of the basal membrane (mb) the cells are very small and resemble oogonia. Their nuclei (n) and mitochondria (m) are both well-preserved.

Hitachi HS-6, 11 200 diam.

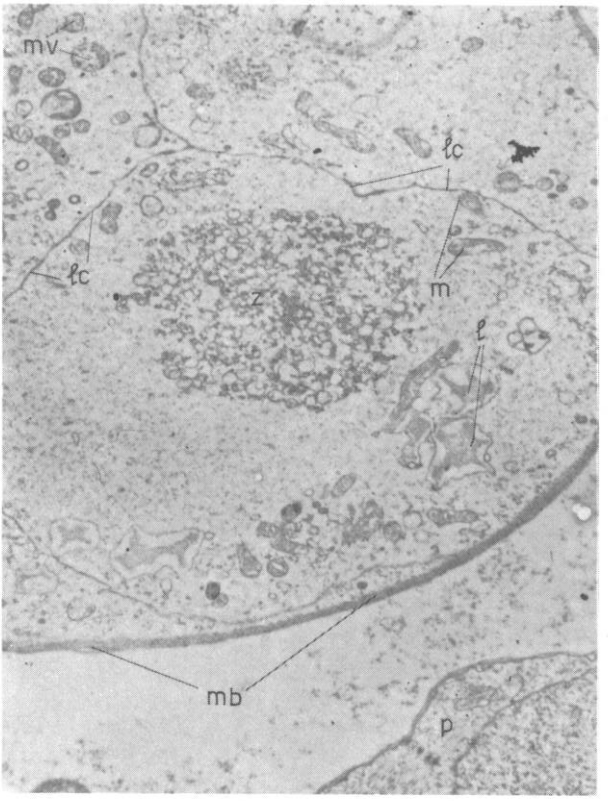

Fig. 15

Electron micrograph of a more distal level of the ovariole in the subject of Fig. 14. Inside the peritoneal membrane (p) and basal membrane (mb), some oogonia can be seen in which the cellular membranes (lc), some mitochondria (m) and multivesicular bodies (mv) seem to be perfectly preserved, whereas many other organelles are grouped in necrotic clumps (z) and vacuoles full of lipids (l) appear. Hitachi HS-6, 11 200 diam.

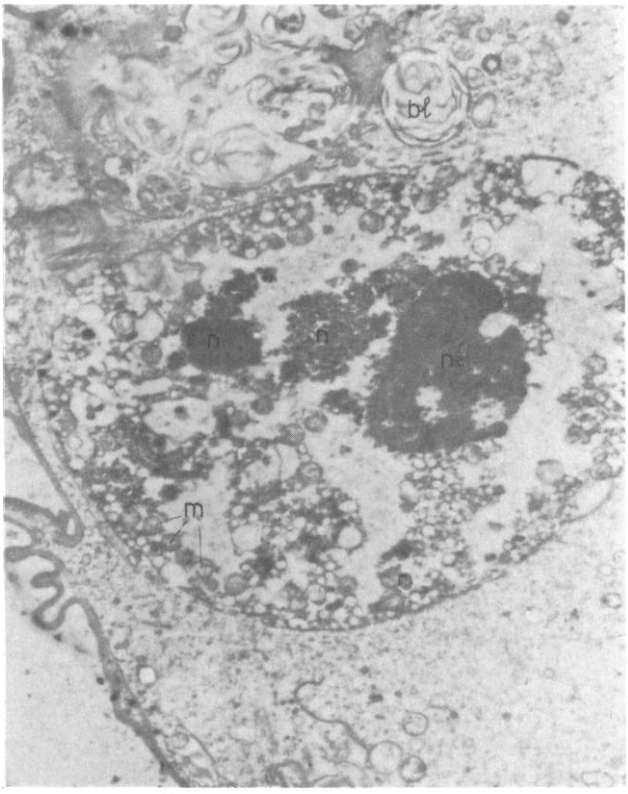

Fig. 16

Region of heavy damage at another level of the ovariole in the subject of Fig. 14 The cellular membranes (lc) seem to be often interrupted, while all the cytoplasmic organelles are accumulated in necrotic clumps.
Hitachi HS-6, 11 200 diam.

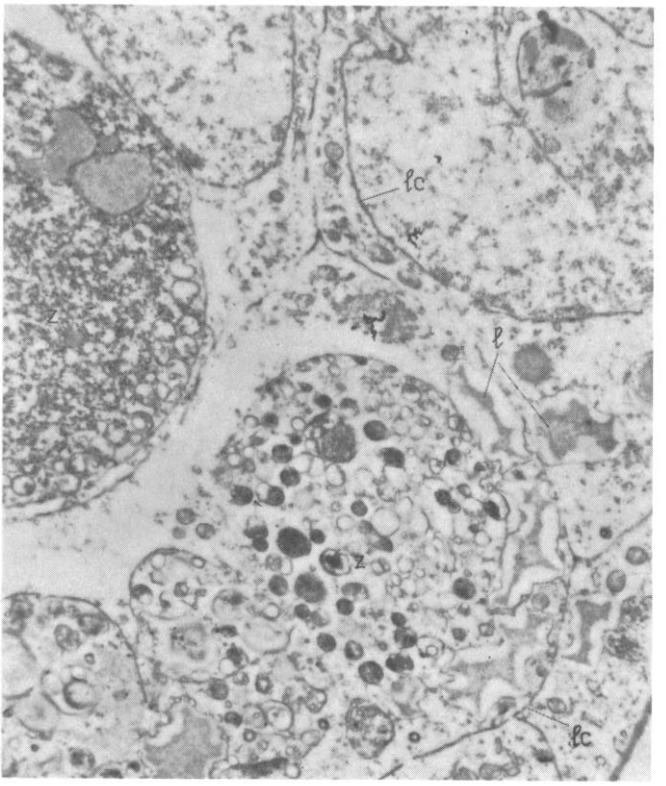

Fig. 17

Cell in karyorrhexis in the ovariole of an adult Dacus oleae Gmel. female, irradiated with 5 kr at the pupal stage

The mitochondria (m) and the other cytoplasmic organelles are accumulated in clumps, while the nuclear material (n) seems to have gathered into thick osmiophile clots. In the surrounding cells many organelles, including some lamellar bodies (bl), appear to be well-preserved.

Hitachi HS-6, 11 200 diam.

Fig. 18

Apex of an ovariole of the subject of Fig. 17
The only visible cell in which the organelles (mitochondria (m) and ergastoplasm (e)) seem to be well-preserved is surrounded by a thick involucrum of collagen fibrillae (c), with distinct regular markings.
Hitachi HS-6, 11 200 diam.

Fig. 19

Apex of the ovariole in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 12 kr The cells all show signs of morbidity in the nuclei (n) and in the cytoplasm, where the mitochondria (m) seem rather dense and where frequent lipidic deposits (l) appear. The cells are isolated from each other and surrounded by a thick texture of collagen fibrillae (c).
Hitachi HS-6, 11 200 diam.

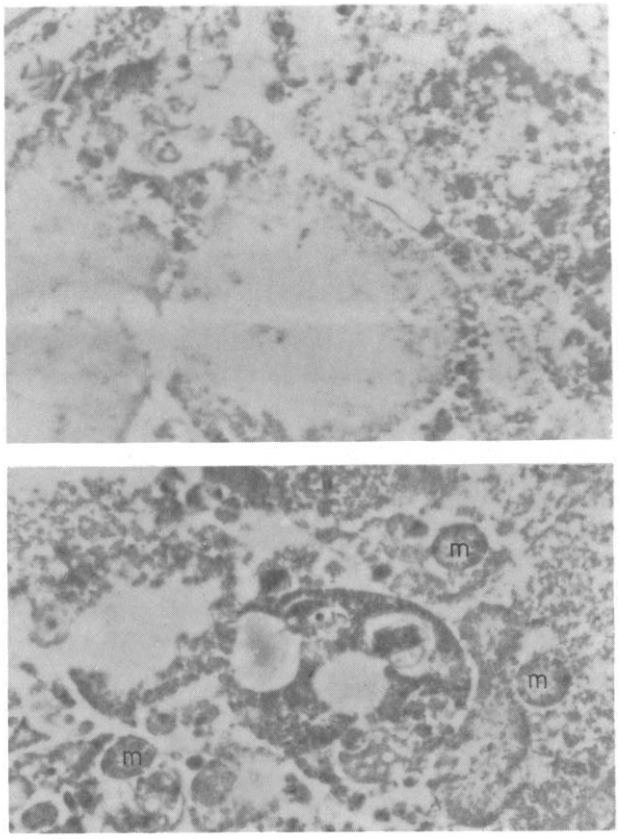

Fig. 20

Portions of cytoplasm in the ovarioles of an adult Dacus oleae Gmel. female irradiated with 20 kr at the pupal stage
Some degenerating mitochondria (m), can be seen, but the other organelles are unidentifiable.
Hitachi HS-6, 11 200 diam.

extent and show frequent vesiculations, leading to the formation of ergastoplasmic cysts from the outer layer, which is constantly very rich in ribosomes.

Oocytes and nurse-cells form groups of 16 units in each ovarian chamber. Of the 16 cells, only one evolves into a mature oocyte ready for fertilization; the remainder have a trophic function and stay between the oocytes themselves, conditioning the meroistic polytrophic type of the ovariole.

The size of the oocyte varies according to the phase of development of the egg. The oocyte (Fig. 2) is characterized, during the prophase, by a large (about 15 μm in diameter), distinctly achromatic, spheroidal nucleus, which is obviously diploid; this nucleus is clearly distinguishable from the polyploid nuclei of the nurse-cells (Fig. 1). The cytoplasm is seen under the optical microscope to be basophile and distinctly granular. During the development of the eggs it becomes charged with yolk spherules and its size increases enormously, while in the nucleus the compact group of chromosomes of the meiotic metaphase is blocked to await the continuation of meiosis that is caused by fertilization.

Under the electron microscope (Fig. 12) the oocyte in prophase is also well characterized by the achromatic appearance of the nucleus, which is transparent to the electrons in almost the whole of its surface, apart from a few accumulations of nuclear material.

The nuclear envelope seems to be intact throughout its length, to have very few pores and to be unconnected with the ergastoplasmic cysts.

The surrounding cytoplasm is very rich in organelles, especially large spheroidal mitochondria with moderately distended internal cristae. One also observes some lamellar bodies, lysosomes and masses of ribosomes, but ergastoplasm in its typical appearance is not found. The cell-boundaries of the oocytes are well squared and often have comparatively wide openings (as much as 2-3 μm) letting accumulations of substance (mitochondria and ribosomes) pass through from the surrounding nurse-cells. The cell membrane around the openings is perpendicular to the cell wall and looks like large rings (Fig. 12).

The nurse cells in full development have a very large spheroidal nucleus (reaching 10-12 μm in diameter) quite rich in DNA. In earlier studies [25] it was demonstrated that this value would correspond to 16-ploidy, although the structure of the chromosomes (Fig. 1) suggests that these cells are polytene. There is a single very large nucleolus, and the cytoplasm, seen under the light microscope, is homogeneous and rather rich in RNA.

Under the electron microscope (Fig. 10) the nurse-cells are seen to be bounded by straight membranes, almost completely without bends, often with quite wide openings at several points and communicating, either directly or by vesiculation and membrane flow effects, with neighbouring cells (trophocytes, oocytes or follicular cells). The richness of organelles (Fig. 11), consisting of large spheroidal mitochondria with few internal cristae, multivesicular and multilamellar bodies and small Golgi fields, is considerable. Sometimes large ergastoplasmic cristae are present, but more often the ribosomes are free and always extremely plentiful. These, together with mitochondria, pour into the oocytes through the large openings of the plasma membrane. The nucleus is filled with granular chromatin,

often in clumps; also the polytene, strongly osmiophilic chromosomes, looking like thick strings, can sometimes be seen, always in pairs. The nucleus is spheroidal and homogeneous. The nuclear envelope communicates with the outside through numerous pores with annuli; from its outside layer small chains of ribosomes originate which then separate into the cytoplasm, while the vesiculations with formation of ergastoplasmic cysts, which are characteristic of follicular cells, seem to be absent.

(B) Structure of the irradiated ovary

Females irradiated at the pupal stage with the lowest doses of irradiation (2, 4 and 5 kr) are found on emergence from the puparium to have very small ovaries bearing no eggs. At the lowest dose, a few mature oocytes may be found in the oviducts, but they are always malformed and have very little yolk. The ovarioles (Fig. 4) have a peculiar appearance: they show, throughout their length, inside the peritoneal sheath, an assemblage of non-differentiated cells, heaped up in disorder, that call to mind the oogonia in the germarium. These cells have diploid nuclei, with DNA accumulated in short, thick sticks; the cytoplasm is very sparse and poor in RNA. Mitosis is completely absent. In many cases the DNA is accumulated in one homogeneous drop, and later comes out by rupture of the nuclear envelope. One has, in other words, frequent pyknosis followed by karyorrhexis.

Less usually, small groups of cells tend towards a kind a maturation, i.e., the cytoplasm is slightly enriched in RNA, whereas the nucleus has not undergone any variation, either by becoming richer in DNA (as in the case of nurse and follicular cells), or by undergoing meiosis (as in the case of the oocytes).

Even under the optical microscope, these cells, and particularly those at the pointed end of the germarium, are seen to be sparsely distributed and separated from each other, and that compact mass of oogonia which characterizes the distal zone of normal ovarioles is no longer identifiable.

Investigation under the electron microscope confirms this picture.

In the whole ovary the greatest part of the tissue is constituted by peritoneal membranes, which preserve a regular structure (Fig. 14) and which, since they contain a much smaller organ than the normal one, are convoluted. Of the cells forming the ovarioles, the better-preserved ones show, as we have already seen under the light microscope, a structure quite similar to that of the oogonia: that is, they contain (Figs. 14 and 15) small mitochondria rich in cristae and small cysts of ergastoplasm, but very few free ribosomes. The nucleus shows chromatin normally distributed in granules, but it constantly shows signs of damage in the karyotheca, which is frequently seen to be interrupted for long stretches and to have lost the power of producing ergastoplasmic cysts.

Sometimes, though rarely, homogeneous, osmiophilic drops appear in the cytoplasm; they seem to be of a lipidic nature. In many cases (Figs. 16 and 17), the damage is more accentuated: the mitochondria, always slightly more opaque than in normal cases, contract and degenerate into homogeneous vesicles which are intensely osmiophilic; the lipidic drops increase in number and size; the free ribosomes tend to coalesce into granu-

lar, opaque clumps; the ergastoplasmic cysts and the Golgi fields dilate into spherical, empty vesicles.

The whole of the morbid organelles come together in degenerating masses displaced into the middle part of the cell(Fig. 15), while the remaining part of the cytoplasm contains only the submicroscopic trabecolatum. For its part, the nucleus degenerates with similar phenomena. Nuclear and nucleolar material gather separately and form large drops on the inside of the remains of the karyotheca, in which the normal exchanges with the cytoplasm are suspended; meanwhile breaks become more and more frequent. Mixed with these cells are some richer in ribosomes and ergastoplasm, but always with a small, diseased nucleus. Along the entire length of each ovariole, the basal membranes seem more compact and also slightly thicker than in normal subjects. However, the distal part (i.e., that of the germarium) presents a quite special picture (Fig. 18); it is surrounded by an enormous accumulation of collagen fibrillae identical with those which constitute the normal basal membrane, twisted up in several layers all round the cells and intruding between them in such a way as to separate them. The distal area of the ovariole is thus held together only by the accumulation of collagen fibrillae, which in normal subjects is much sparser and constitutes only the basal membrane. The direct inter-cell relations seem to be lost, but the extent of morbidity in the cells of this area is rather limited; they fall into the category of moderately damaged oogonia.

With doses of 8, 10 and 12 kr the damage to the ovaries is greatly increased. These are now tiny (they measure on the average $35 \times 15 \times 15\ \mu m$), are pyriform in appearance and have a very limited amount of cells, almost all degenerating. The peritoneal membrane appears, as usual, intact and constitutes the greatest part of each ovary; the ovarioles (Fig. 5) contain only a few cells (all of them can be regarded as degenerating oogonia) with sparse, Brachet-negative cytoplasm, and nuclei often in pyknosis or in karyorrhexis.

Under the electron microscope (Fig. 19), the integrity of the peritoneal membrane is confirmed, and one observes lesions of the oogonia of the same type as those described for the smallest doses, but becoming progressively more acute and serious as one proceeds from 8 to 12 kr. The characteristic increase of collagen fibrillae around the ovarioles is even more marked than in the cases described above, and the cells contained in the collagen mass seem to be small and degenerating. The cell boundaries and the nuclear envelope are often broken for considerable stretches and show no signs of exchange of substances; the mitochondria are small, dense and sometimes unidentifiable; ergastoplasm is almost entirely absent, whereas coarse lipidic inclusions in large vacuoles are abundant. The nuclear material is diffused in minute granules, often against the karyotheca, and shows no signs of a nucleolus.

At a dose of 20 kr, the damage to the gonads is even more accentuated. The ovaries are even smaller than in the cases just described and are reduced almost to nothing but empty peritoneal membranes (which appear, even under the electron microscope, perfectly preserved) whereas the oogonia have almost all degenerated. In fact one observes (Figs. 6 and 7) very few groups of cells along each ovariole, with almost always a nucleus in pyknosis

or karyorrhexis and very poor in cytoplasm, which seems to be stained by pyronin only in small, extremely concentrated drops.

Under the electron microscope (Fig. 20) the various cellular constituents are unidentifiable; the nuclear envelopes are dissolved and the nuclear content comes out in drops; the mitochondria are dense, granular and broken, tightly packed, by a homogeneous and granular substance which is difficult to identify, near the cell membranes. One recognizes some multilamellar bodies, quite altered, but on the whole the cells seem to be in necrosis.

At 30 kr the effect is even more accentuated. The ovaries are tiny (about $18 \times 12 \times 12$ μm) and very poor in cells (Fig. 8) on the inside of the peritoneal membranes, which are still intact. All of the oogonia present seem, without exception, to be in pyknosis or in karyorrhexis and present a cytoplasm which, under the optical microscope, seems concentrated in thick, strongly basophile drops, and under the electron microscope none of the cellular components is identifiable.

DISCUSSION

The results presented here lead us to the conclusion that the damage caused by ionizing radiation to the ovarian structures of Dacus oleae Gmel. irradiated at various doses at the pupal stage is qualitatively similar but variable in intensity.

The ovarioles always become extremely small, without mitosis and poor in cells, which are constantly blocked at the oogonial stage and are thus not permitted to differentiate into oocytes, nurse-cells and follicle cells. Thus there remains only one type of germinal element, the oogonia, which undergo alterations of all the cellular elements. The nucleus shows considerable pyknosis and karyorrhexis effects, nucleolar thickening and rupture of the karyotheca, which loses its contacts with the ergastoplasm; the cytoplasm is poor in ergastoplasm and shows few, small and thickened mitochondria but abundant lipidic inclusions, while the exchange with neighbouring cells becomes extremely poor or almost absent; in the case of extreme damage, large areas of cytoplasm seem to be necrotic, and the organelles, which are condensed into compact clumps, are unidentifiable. The single ovarioles are surrounded by a stroma of enormously thickened collagen fibrillae. The peritoneal membrane cells, on the other hand, do not show any alteration, even at the most powerful doses tested.

On the whole the alterations we have described generally agree with what has been reported on this subject in the literature, on both optical microscope studies in mammals and insects and electron-microscope investigation in mammals; thus, the nuclear lesions in the mouse described by Parsons are perfectly comparable with those that we have observed, even to the alterations of the mitochondria, the decrease of ribosomes, the accumulation of lipids, etc.

One particularly interesting observation that has emerged from our research is the blocking of the expulsion of ergastoplasmic vesicles from the outer layer of the nuclear envelope; this alteration had been observed earlier in the cells of the intestinal epithelium of irradiated Dacus oleae [23],

and the reason it is not reported in the descriptions of other authors (e. g. PARSONS [20]) is probably that a smaller amount of ergastoplasmic substance was contained in the cells they studied in comparison with the cells we have examined.

Another interesting observation is the accumulation of collagen fibrillae round each ovariole in such quantity as to cause the separation of whole groups of cells or of single cells.

This increase of collagen fibrillae, which is commonly observed in irradiated tissues, has already been described less fully in relation to the ovaries of mammals, but we have not found any reference to it in relation to the study of the ovaries of irradiated insects by optical microscope. Thus the electron-microscope observations represent completely new information.

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DISCUSSION

Mrs. M.-H. BINGGELI: In one of the slides you showed during your oral presentation I observed a number of well-defined patches in the cytoplasm, as seen in section in the electron microscope. You showed us some drops which you referred to as lipid, and I think you referred to some mitochondrial changes, whereas I am thinking of certain rather diffuse patches in the cytoplasm. You said they were due to radiation damage, and I was wondering whether there were any structural components as well?

B. BACCETTI: Yes, in certain cases, though not for the first irradiations, you do get, especially in the case of damage, certain substances diffused in the cytoplasm, owing, I think, to a rupture, shall we say, of the vessels or cellular membranes. These substances showed up in my illustrations as necrotic areas in the central part of the cytoplasm, and this is what you saw. I would stress that this is something quite different from the mitochondrial and lipidic degeneration, which is the most classical of all known lesions, not only in radiobiology but also in the general pathology of mammals and of man.

W. KLOFT: Having had personal experience in the pathology of holometabolic insects, I should like to ask whether the degeneration of the ovaries that was described could not be direct as well as indirect radiation damage. As I was able to show, the developing ovary of a pupa which is damaged in any way is, so to speak, the insect's weakest point. It degenerates easily since it is not an organ necessary to survival. One should therefore always consider also the more indirect radiation effects of ovaries in disturbing any other systems within the irradiated insect.

B. BACCETTI: I was referring to a previous study I carried out on the middle intestine of the same insect at the same irradiation doses. I always make observations on other somatic tissues besides the peritoneal membranes, and it can be said that for the higher irradiation doses, 20 or 30 kr, total damage does occur. For 10 kr, i.e. in almost all the slides I showed you, there is no total damage to the most sensitive tissues. The major part of the lesions shown are directly on ovaries and it is only at higher irradiation doses that there may be indirect damage, which is not very important, however, since it is the same type of damage as we saw in connection with the first dose.

P. NARDON: When you irradiate pupae, do you ever observe in the adults any decrease in the number of ovarioles, or ovarioles with a double germarium? I ask this because I have observed such phenomena in the progeny of the grain weevil which I irradiated with X-rays.

B. BACCETTI: I have never observed any difference in the number of ovarioles, but I must say that I did not look for it. I simply made peripheral observations, since the methods of fixation I had to use are expensive and I could not undertake a microscopic study. From what I saw I think there is no great difference in the number of ovarioles, especially as the peritoneal membranes are absolutely normal and consequently the envelope, shall we say, of each ovariole is completely preserved. Their number did not, I think, diminish in any way, mainly because irradiation took place with the number already established at the pupal stage.

STERILIZATION OF DACUS OLEAE BY GAMMA RADIATION

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Abstract — Résumé — Аннотация — Resumen

STERILIZATION OF DACUS OLEAE BY GAMMA RADIATION. Three developmental stages of the olive fly were exposed to gamma radiation from a Co^{60} source and the sterilization dose was determined. For the fourth-instar larvae the sterilizing dose was 2000 ± 300 rad, for the pupae 11 000-15 000 rad and for the adults 15 000-18 000 rad. Mating studies showed that the sterility lasted throughout the life of the adults.

The effect of dose on emergence was studied with fourth-instar larvae and on the pupae at daily intervals. The larvae were exposed to 300-3000 rad at 300-rad intervals, while the pupae were exposed to 3000-15 000 rad at 3000-rad increments. Maximum emergence was obtained when eight-day-old pupae were irradiated at the above-mentioned dose-rates. Significant fluctuations occurred at all dose-rates.

STERILISATION DE LA DACUS OLEAE PAR IRRADIATION GAMMA. On a exposé aux rayons gamma d'une source au ^{60}Co des mouches de l'olive se trouvant à trois stades de développement, et on a déterminé la dose nécessaire à la stérilisation. Au quatrième stade larvaire, la dose de stérilisation était de 2000 ± 300 rad; pour les pupes, elle était de 11 000 à 15 000 rad; pour les adultes, elle se situait entre 15 000 et 18 000 rad. Des études sur la reproduction ont montré que la stérilité persistait pendant toute la durée de vie des adultes.

On a aussi étudié l'effet de la dose au quatrième stade larvaire et sur les pupes, à des intervalles de un jour. Les larves ont été exposées à des rayonnements de 300 à 3000 rad, la dose augmentant de 300 rad par jour, tandis que les pupes recevaient des doses de 3000 à 15 000 rad, l'augmentation étant de 3000 rad chaque fois. On a obtenu l'éclosion maximum en irradiant des pupes âgées de huit jours avec les doses mentionnées plus haut. Toutes les doses ont entraîné des fluctuations sensibles.

СТЕРИЛИЗАЦИЯ DACUS OLEAE ГАММА-ОБЛУЧЕНИЕМ. Три группы оливковых мух в различных стадиях развития подвергали гамма-облучению Co^{60} и определяли дозу стерилизации. Для четвертой возрастной стадии личинок доза стерилизации оказалась равной 2000 ± 300 рад, для куколок 11 000 - 15 000 рад и для взрослых особей 15 000 - 18 000 рад. Изучение мужских особей показало, что у взрослых особей стерильность сохраняется в течение всей жизни.

Изучалось влияние доз на выплод для четвертой возрастной стадии личинок и куколок с интервалом в один день. Личинки подвергались облучению в 300 - 3000 рад с интервалами в 300 рад, тогда как куколки облучались при 3000 - 15 000 рад с инкрементом в 3000 рад. Максимальный выплод был получен, когда восьмидневные куколки подвергались облучению при вышеупомянутых мощностях доз.

Значительные колебания наблюдались при всех мощностях доз.

ESTERILIZACIÓN DE DACUS OLEAE POR IRRADIACIÓN GAMMA. El autor ha sometido tres fases de desarrollo de la mosca del olivo a las radiaciones gamma de una fuente de ^{60}Co, determinando la dosis esterilizadora que fue de 2000 ± 300 rad para las larvas del cuarto estadio, de 11 000 a 15 000 rad para las ninfas y de 15 000 a 18 000 rad para los adultos. Al estudiar las relaciones intersexuales se observó que la esterilidad perdura durante toda la vida de los adultos.

Todos los días se estudiaron los efectos de la dosis sobre la transformación de larvas del cuarto estadio y de ninfas. Las larvas fueron expuestas a dosis de 300 rad que se fueron aumentando de 300 en 300 rad hasta llegar a 3000, y las ninfas a dosis de 3000 rad que se fueron aumentando de 3000 en 3000 rad hasta llegar a 15 000. El porcentaje máximo de transformación se obtuvo irradiando ninfas de ocho días de vida con las dosis mencionadas. Para todas las intensidades de dosis se observaron fluctuaciones importantes.

INTRODUCTION

The control of harmful insects has occupied many a scientist's time. In some cases, efforts to control some of them with insecticides have proved unsatisfactory. However, research on the control of some harmful insects by irradiating them with Co^{60} has been promising and in a few cases, such as with Callitroga hominivorax [1, 2, 3] in Curaçao and Florida [4, 5], the results have been quite satisfactory.

This success induced the Greek Atomic Energy Commission to study the possibility of the control of Dacus oleae by Co^{60} irradiation. This study is of supreme importance in the economy of Greece.

The control of Dacus oleae Gmel. presents more problems than that of Callitroga, because it is a polygamous insect and has four to five annual generations. To alleviate these difficulties it has been suggested that a large number of sterilized males be released, a successful method which has brought positive results in laboratory and field experiments conducted by the Hawaii Fruit Fly Laboratory of the United States Department of Agriculture, on Dacus dorsalis Hendel, Ceratitis capitata Wied and Dacus cucurbitae Coq.

The aim of the present investigation was to determine the correct sterilizing dose and its effect on the emergence, vitality and fertility, and also on the resistance, of Dacus oleae to Co^{60} irradiation with the advancement of age. A similar experiment has been carried out in Italy [6].

The life-cycle of Dacus oleae consists of the three stages larva, pupa and adult. The female insects oviposit in the olive fruit, where the larva develops, feeding on the mesocarp. Pupation takes place either in the larval gallery in the fruit or, especially in the late-Autumn generations, in the soil. The duration of the larval and pupal stages varies, depending, among other factors, on temperature. In our laboratory, at 25°C, the larval life was about 12 d and the pupal life also about 12 d.

MATERIALS AND METHODS

The insect material used was taken from infested olive fruit sent to us from several areas of the country, mainly Patras and Chania, which we held at a temperature of 26 ± 1°C and a relative humidity of 70 ± 5%.

Larvae were irradiated during the period between their exit from the fruit and the formation of the puparium. Each group of irradiated larvae consisted of 20-30 individuals. There were three replications of each test in each season. The tests were repeated in three different seasons. Thus, 180-200 larvae were used in determining the effects of each radiation dose.

Pupae were irradiated 1 to 9 days after pupation. Each group of irradiated pupae consisted of 100 individuals. Repetitions were conducted in the same way as in the larvae. About 900 insects were used in determining the effects of each radiation dose.

Adults were irradiated only on the day of emergence, after they had fed on an adult liquid food kindly given to us by Dr. K. S. Hagen. Each group of irradiated insects consisted of 40 individuals. Repetitions were conducted in the same way as in the larvae and pupae. About 360 insects were used in determining the effects of each radiation dose.

The irradiation was done at a 550-c Co^{60} source. The total dose varied between 330 and 18 000 rad, depending on the developmental stage of the insect, at dose-rates of 330 rad/min and 600 rad/min, with 2% accuracy. Groups of 30 or more insects were irradiated in small test tubes stoppered with cotton-wool at approximately 20°C. The irradiated insects were maintained at 25°C and 70% relative humidity in Plexiglass insect cages.

After irradiation, all the adults, including those originating from irradiated larvae or pupae, were offered the above-mentioned liquid food.

Two days after emergence, the separation of males from females took place. The irradiated males were put with normal females and vice versa. In each cage, paraffin domes were placed for oviposition. A water-soaked cotton-wool wad was inserted in each dome to prevent the eggs from drying out.

The fertility and fecundity were determined by counting the eggs laid and finding the percentage of hatching. It was not possible to follow up the further development of the newly hatched larvae and subsequent stages because of lack of a satisfactory artificial larval food medium.

The eggs collected each day from the paraffin domes were placed on absorbent paper soaked in distilled water in a Petri dish.

At 25°C hatching occurred within the first two days after the eggs had been put in the Petri dish. The mating ability of the irradiated males or females was determined by ascertaining the presence of spermatozoa in the spermatheca of the females.

The number of adults emerged in each case was recorded as a percentage of the number emerged in the controls.

PRELIMINARY RESULTS

Irradiation of larvae

The results are shown in Table I and Fig. 1. The figures on the percentage of adult emergence from irradiating larvae is the average of three groups, each including approximately 25 individuals. Doses above 1650 rad caused sterility in both sexes, without obviously affecting the mating habits or the longevity of the adults, although on an average irradiated individuals had a somewhat shorter adult life than normal ones.

In females from irradiated larvae, the ovaries were atrophied: there was a complete absence of ova. Insects placed with normal males showed a very small number of spermatozoa in their spermathecae.

In males from irradiated larvae, live spermatozoa were observed in their testes; however, their premating period was extended to 10 or more days on an average against 1-2 days in the normal males.

Irradiated pupae

The results are shown in Table II and Figs. 2 and 3. Figs. 4-9 show the tolerance of pupae to radiation with increasing age.

TABLE I

EFFECT OF GAMMA IRRADIATION ON FULL-GROWN LARVAE OF DACUS OLEAE IN RELATION TO ADULT EMERGENCE
(Larvae fed on olive fruit)

Total dosage (rad)	Adults emerged (corrected) (%)	Fertile females (%)
330	95	60
660	74	48
990	82	30
1320	83	15
1650	36	3
1980	29	0
2310	23	0
2640	17	0
2970	14	0
3300	12	0

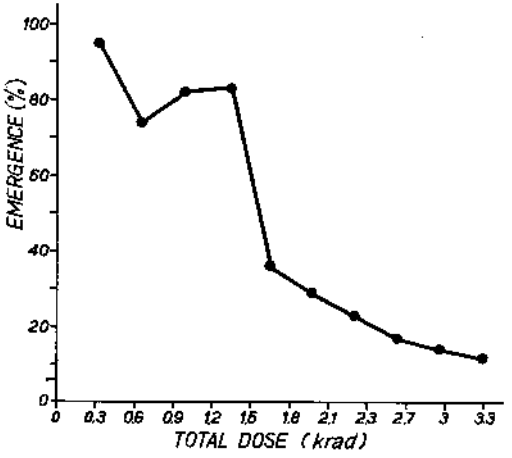

Fig. 1

Irradiation of larvae

Females (Observations December 1961 - February 1962)

Their mating ability was not impaired. The females which had emerged from pupae irradiated with 9, 12 and 15 krad showed, from the tenth day after their emergence up to their natural death:

(a) Spermatozoa in their spermatheca, like those of the control group.
(b) Completely formed eggs, of about the same number as those of the control group.
(c) A greatly prolonged preoviposition period as compared with that of the control group.

TABLE II

EFFECT OF GAMMA IRRADIATION ON PUPAE OF DACUS OLEAE OF VARIOUS AGES IN RELATION TO ADULT EMERGENCE
Samples from different parts of the country
(December 1961 - February 1962)

Total dosage	Adult emergence (corrected) (%) after irradiation of pupae at (d)					
(krad)	4	5	6	7	8	9
3	30	36	69	83	85	107
4.5	8	9	51	70	100	86
6	0	0	72	99	83	86
9	17	12	81	76	73	86
12	9	80	79	55	67	40
15	0	50	89	118	109	101

TABLE III

EFFECT OF GAMMA IRRADIATION ON VARIOUSLY AGED PUPAE OF DACUS OLEAE IN RELATION TO ADULT EMERGENCE
Samples from one part of the country
(May-June 1962 and October - December 1962)

Total dosage	Adult emergence (corrected (%) after irradiation of pupae at (d)			
(krad)	5	6	7	8
3	42	44	69	72
4.5	11	33	58	85
6	10	71	84	75
9	11	77	64	66
12	44	50	64	60
15	44	52	76	82

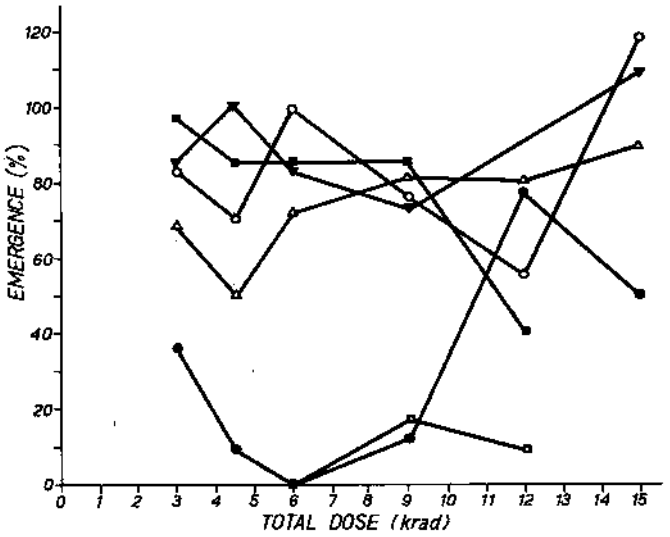

Fig. 2

Irradiation of pupae

□ 4 d old ○ 7 d old
● 5 d old ▼ 8 d old
△ 6 d old ■ 9 d old

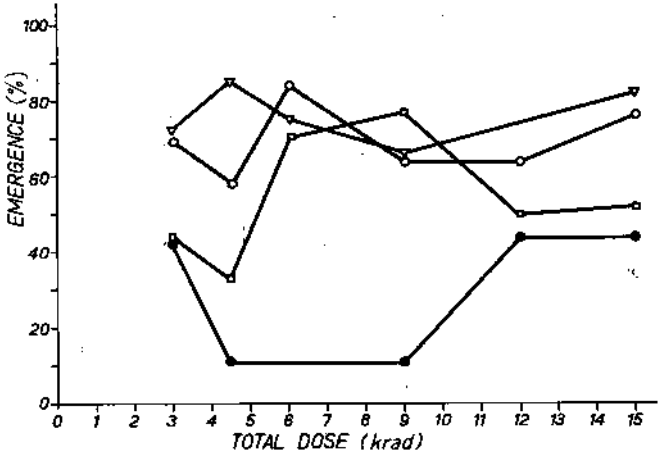

Fig. 3

Irradiation of pupae

● 5 d old ○ 7 d old
□ 6 d old ▽ 8 d old

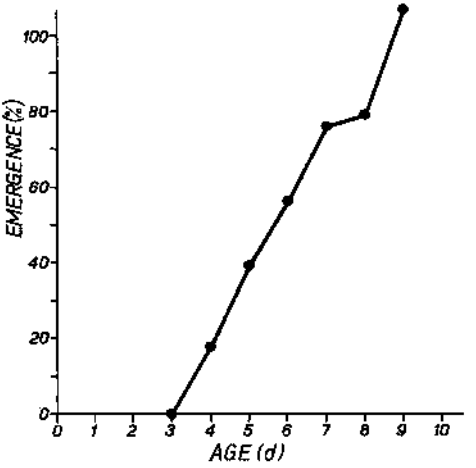

Fig. 4

Tolerance of pupae to 3 krad

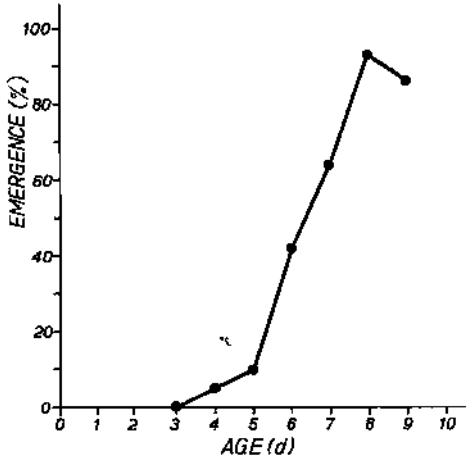

Fig. 5

Tolerance of pupae to 4.5 krad

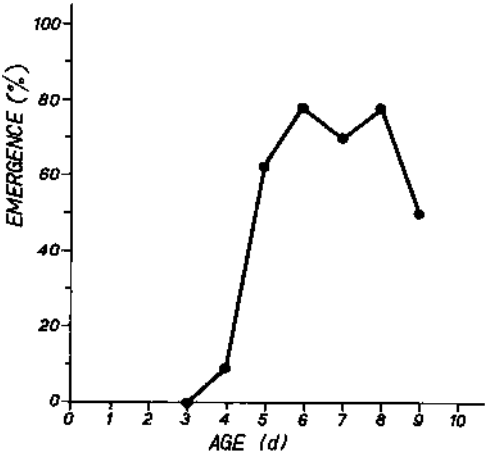

Fig. 6

Tolerance of pupae to 6 krad

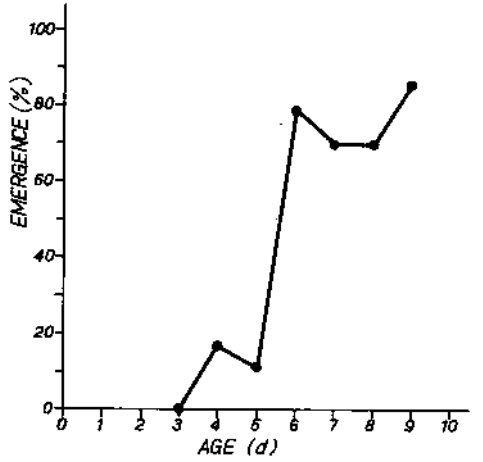

Fig. 7

Tolerance of pupae to 9 krad

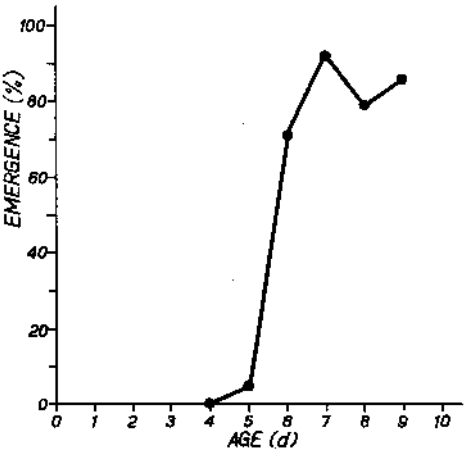

Fig. 8

Tolerance of pupae to 12 krad

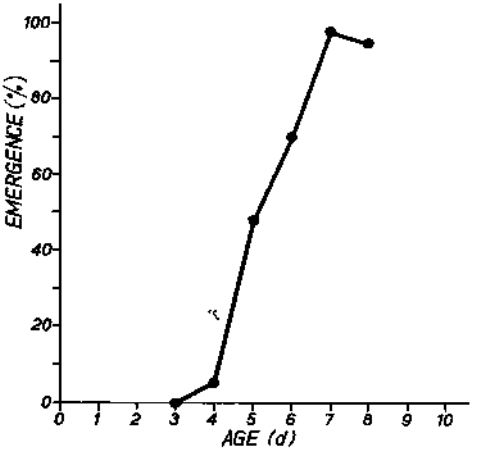

Fig. 9

Tolerance of pupae to 15 krad

Females (Observations May-June 1962 and October-December 1962)

Females from pupae irradiated at doses of 12 and 15 krad showed a great number of spermatozoa in the spermathecàe but atrophied ovaries with no eggs 25, 30 and 62 d after emergence.

Males (Observations December 1961-December 1962)

The mating ability was not impaired after pupal doses of 12-15 krad but mating occurs 5-10 d* later, depending on the strength of the dose. The normal mating time is 1-2 d* after emergence.

Male fertility was 0-5%, as compared with 70% for the control group (Fig. 10). The density and motility of spermatozoa in vitro was the same as for the control group.

Adults of both sexes emerging from pupae irradiated with 12 and 15 krad lay upside-down in their cages for the first four days after emergence. They subsequently recovered and behaved approximately the same way as irradiated adults.

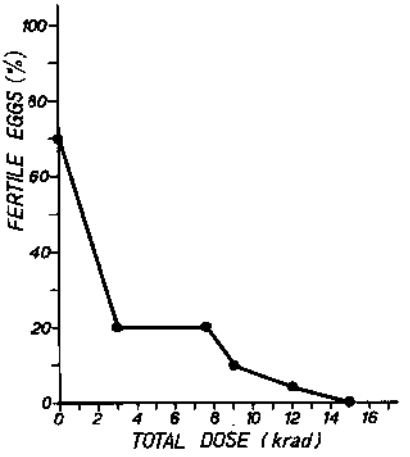

Fig. 10

Male fertility
(Doses 12-15 krad)

Irradiated adults

The results are shown in Table IV. In females, the mating ability was about the same as that of the control group. Fecundity was nil, as no eggs were laid or observed in the ovaries, after doses of 15-18 krad.

In males, mating ability did not seem impaired but mating was delayed by about 10 days. Sterility was complete after doses of 15-18 krad.

*The premating period figures should be increased by 5 to give the actual days on which mating occurred. It was noticed that the females would not accept the males for the first five days after emergence.

TABLE IV

EFFECT OF GAMMA IRRADIATION ON ADULT DACUS OLEAE IN RELATION TO SURVIVAL

Total dosage (krad)	Surviving 3 d after irradiation (%)	
	Males	Females
6	65	70
9	75	86
12	93	97
15	80	90
18	72	80

The length of the adult life after small doses (3-6 krad) was not different from that of normal adults. However, with increasing dosage the adult life was considerably shortened [7].

CONCLUSIONS

Larvae

The adults (male and female) which emerged from larvae irradiated with 2000 ± 300 rad remained sterile throughout their adult life (85-90 d). This dose seems to be satisfactory. Larvae irradiated with 2970-3300 rad developed into pupae which died as such before adult emergence.

Pupae

This stage is the most suitable and easy to handle for irradiation purposes. Adults from pupae irradiated with 12-15 krad remained sterile throughout their life. This stage was selected because the testes can be distinguished and the progress of spermatogenesis can be satisfactorily observed. In the testes of normal pupae, all stages of spermatogenesis except the mature spermatozoa are apparent on the fourth day after pupation and on the fifth day all stages, including the mature one, are apparent. On the sixth, seventh and eighth days the number of mature spermatozoa increases steadily.

Adults

It seems desirable to feed adults before irradiation, since those irradiated before they were fed had a high mortality (about 50%). Adults irradiated with 15-18 krad remained sterile throughout their life.

Sensitivity to radiation differed between insects from different parts of the country.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to Professor G. Pantazis for the suggestion of this problem and his advice during the course of this experiment.

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DISCUSSION

B. BACCETTI: I am happy to learn that my Greek colleagues have studied the problem of possible Dacus control by the method of sterilized males. These results confirm perfectly the findings I myself obtained in Italy, which were published two years ago. It has already been shown in Italy that it is possible to control the Dacus with irradiated males and we have also studied the vitality of the adults at the different irradiation doses, not only on the basis of adult emergence, but also using an electron microscope on particularly sensitive somatic tissue.

P.S. ORPHANIDIS: I would like to ask whether there was a difference in the duration of the pupal stage between pupae from sterilized, third-stage larvae and those from non-sterilized larvae.

Mlle. H. THOMOU: No, we did not observe any difference.

EFFECTS OF VARIABLE DOSE-RATES ON RADIATION DAMAGE IN THE RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM HERBST

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF VARIABLE DOSE-RATES ON RADIATION DAMAGE IN THE RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM HERBST. In order to find out whether a change in dose-rate would significantly alter a radiation response, eggs as well as the adults of Tribolium castaneum were tested for viability and fertility respectively after exposure to gamma radiation at different dose-rates. The doses employed were 2000 and 5000 rad and the dose-rates ranged from 126 rad/h to 140 000 rad/h. It was observed that with an increase in dose-rate there was a decline in the fertility of the adults. Similarly the viability of the eggs was considerably reduced as the dose-rate increased, but at very high dose-rates there was a significant increase in viability. The significance of these findings is discussed.

EFFETS DU DÉBIT DE DOSE SUR LES DOMMAGES RADIOINDUITS CHEZ LE TÉNÉBRION ROUX (TRIBOLIUM CASTANEUM HERBST). En vue de déterminer si une variation du débit de dose modifie de manière significative la réponse aux rayonnements, on a fait des essais sur des œufs et des adultes de Tribolium castaneum, dont on a étudié respectivement la viabilité et la fécondité après exposition à des doses de rayons gamma d'intensités différentes. On a administré des doses de 2000 et 5000 rad, le débit de dose variant de 126 à 140 000 rad/h. On a constaté que la fécondité des adultes diminuait à mesure que le débit de dose augmentait. De même, la viabilité des œufs était considérablement réduite à mesure que le débit de dose augmentait, mais pour des débits de dose très élevés la viabilité augmentait sensiblement. Les auteurs analysent les données obtenues.

ВЛИЯНИЕ РАЗЛИЧНЫХ МОЩНОСТЕЙ ДОЗ НА РАДИАЦИОННОЕ ПОВРЕЖДЕНИЕ КРАСНОГО МУЧНОГО ЖУКА, TRIBOLIUM CASTANEUM (HERBST). Для определения зависимости реакции на облучение от изменения мощности дозы изучались жизнеспособность и фертильность яиц, а также взрослых особей Tribolium castaneum после гамма-облучения при различных мощностях доз. Использовались дозы в 2000 и 5000 рад, а мощность доз изменялась от 126 до 140 000 рад в час. Было замечено, что с повышением мощности дозы понижалась фертильность взрослых особей. Подобным же образом с повышением мощности дозы значительно уменьшалась жизнеспособность яиц, однако при очень больших мощностях доз жизнеспособность заметно увеличивалась. Обсуждается значение полученных данных.

RADIOLESIONES QUE SUFRE EL TRIBOLIUM CASTANEUM HERBST CUANDO SE LE SOMETE A DOSIS DE RADIACIONES DE DIVERSAS INTENSIDADES. Para averiguar si un cambio en la intensidad de la dosis produce alteraciones fundamentales en la radiosensibilidad, se comprobó la viabilidad de los huevecillos y la fertilidad de los adultos de Tribolium castaneum después de exponerlos a dosis de radiaciones gamma de intensidad diferente. Las dosis empleadas fueron 2000 y 5000 rad, y las intensidades oscilaron entre 126 rad/h y 140 000 rad/h. Al aumentar la intensidad de la dosis se produce una disminución de la fertilidad de los adultos. De modo similar, la viabilidad de los huevecillos disminuyó considerablemente al aumentar la intensidad de dosis, aunque vuelve a aumentar de manera notable cuando las intensidades de dosis alcanzan valores muy elevados. La memoria discute el significado de estos resultados.

INTRODUCTION

It has been known for some time that the development of radiation injury is conditioned by a variety of physical and biological factors before, during

and after treatment. Recently, attention has been focused on the role of dose-rate in modifying the extent of radiation damage. RUSSEL et al. [5] and RUSSEL [6] reported that the frequency of mutations from spermatogonia irradiated at a dose-rate of 0.009 r/min was significantly lower than obtained when the dose-rate was 90 r/min. KALLMAN [4] observed that the LD50 of mice of two inbred strains increased with decreasing dose-rate. BANHAM [1] has mentioned that the survival of Tribolium confusum was very much lower at a dose-rate of 4000 rad/h than at 2000 rad/h. JEFFERIES [3] observed in Oryzaephilus surinamensis that by varying the dose-rate from 1600 to 4000 rad/h "doses for 50% reduction, both in survival and progeny, were significantly increased when the dose-rate was decreased".

The above-mentioned studies have indicated that dose-rate can modify the radiation damage in a number of biological systems. Since the minimum effective dose for radiation disinfestation of grain on a commercial scale will be largely conditioned by the intensity at which this dose is delivered, it is necessary that all future evaluation of this dose be determined in relation to the dose-rate of the source in any projected irradiator. The present study was therefore undertaken with some of the available dose-rates obtained in our gamma irradiation facility with a view to finding out the effects of dose-rate on the viability of eggs and also fertility in the adults of Tribolium castaneum. The findings reported here are of a preliminary nature and it is hoped to pursue this problem in greater detail.

MATERIAL AND METHODS

Adult beetles were placed on previously sieved and sterilized wheat flour for oviposition. The eggs collected after 12 h were irradiated on the second day, at which time their age was approximately between 36 and 48 h. The total dose used was 2000 rad, and this was delivered at different dose-rates, viz. 1020, 3520, 47 420, 81 550 and 140 000 rad/h. For each dose-rate 12 replications of 25 eggs each were irradiated in gelatin capsules. Both the irradiated and the control eggs were incubated at 30°C and hatching was scored on the eighth day after irradiation.

Five-day-old pupae were sexed before emergence. The emerged adults were irradiated on the seventh day, after which they were paired. The doses employed were 2000 and 5000 rad. The dose-rates used for 2000 rad were 126, 3050 and 14 000 rad/h and for 5000 rad 236, 3050, 3520 and 140 000 rad/h. Each mating combination consisted of a single pair and 15 such replications were used for each dose-rate. In all cases about 5 g of sterilized wheat flour with 2% yeast formed both the food and the ovipositing medium. Equal numbers of unirradiated insects served as controls. At intervals of 4 d, eggs laid were removed from the tubes and kept separately for hatching. The experiment was discontinued on the 32nd day after pairing. The average number of eggs laid as well as the percentage viability were recorded.

IRRADIATION FACILITY

The Co^{60} sources used in this study were lodged in a pool containing 15 ft of water. The low dose-rates were obtained by irradiating the samples

at different distances from the sources. The samples were irradiated through an aluminium tube 17 ft in length which ensured aeration during irradiation.

RESULTS AND DISCUSSION

An analysis of the data on the viability of the eggs and the induced sterility of the adults at different dose-rates are presented in Tables I and II.

TABLE I

VIABILITY OF TWO-DAY-OLD EGGS OF TRIBOLIUM CASTANEUM IRRADIATED AT DIFFERENT DOSE-RATES

Total dose (rad)	Dose-rate (rad/h)	Viability and S.E. (%)
0 (Control)	-	78.3 ± 1.9
2 000	1 020	55.3 ± 2.6
2 000	3 520	33.3 ± 4.2
2 000	47 420	7.0 ± 1.8
2 000	81 550	24.3 ± 3.1
2 000	140 000	35.6 ± 2.6

TABLE II

EFFECT OF DIFFERENT DOSE-RATES ON THE FECUNDITY AND FERTILITY OF TRIBOLIUM CASTANEUM

Total dose (rad)	Dose-rate (rad/h)	Number of eggs laid/female	Viability and S.E. (%)
0 (Control)	-	299.3	50.4 ± 2.0
2 000	126	235.1	31.4 ± 2.9
2 000	3 050	217.8	25.57 ± 2.002
2 000	140 000	233.0	21.7 ± 1.8
5 000	236	155.1	16.1 ± 3.23
5 000	3 050	105.8	1.054 ± 0.024
5 000	3 520	113.4	1.005 ± 0.028
5 000	140 000	67.5	0

At a high dose-rate of 47 420 rad/h, the viability of the eggs decreased considerably in comparison with what was obtained at dose-rates below this

level. On the other hand, when the dose-rate was further increased, the susceptibility of the eggs to radiation decreased, as is evidenced by the higher percentage of hatching obtained at 81 550 and 140 000 rad/h. (Fig. 1)

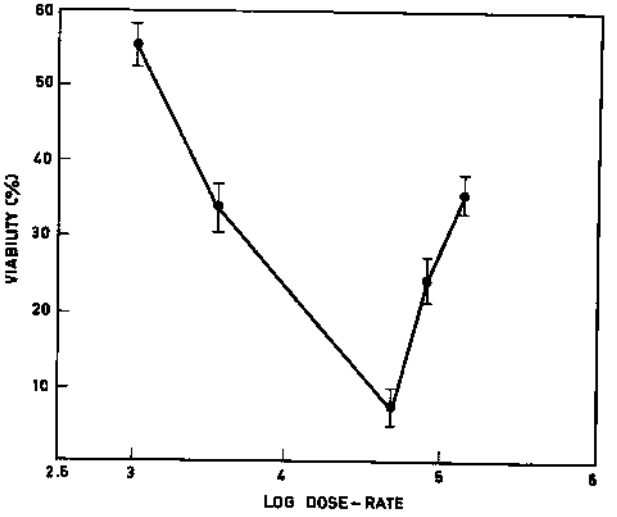

Fig. 1

The effect of different dose-rates on the viability of the eggs of T. castaneum

BULL et al. [2] found in their studies on the susceptibility of the grain weevils to electrons and gamma rays that the sterilizing dose was very much higher in the case of electrons than in the case of Co^{60} gamma rays. This they attributed to the very high dose-rate of the electron accelerator (5.76×10^9 rad/h). SCHWARTZ [7] has shown that at very high doses the extent of radiation damage in maize seeds was less than that observed at lower doses as measured by seedling height. Our results suggest that, as in the case of doses, an inverse relation exists even with dose-rates up to a point beyond which there is an increase in the viability of the eggs. It is apparent from the results obtained in our study that the factors which contribute to an increased viability at low dose-rates are different from those which also increase the viability at high dose-rate. The increased viability at low dose-rates is probably due to the phenomenon of repair taking place in the cells. That this is so has been confirmed by other workers on mice [4, 5].

The way in which very high dose-rates increase survival is at the present moment only a matter of conjecture; presumably it must be brought about by the interplay of several factors. If one assumes that for the manifestation of maximum radiation damage the irradiation period is a critical factor, then any irradiation time greater or less than this fraction might produce less damage in the system. Under these conditions the extent of damage at the above-mentioned high dose-rates can be expected to be less. It is also likely that at very high dose-rates relatively anoxic conditions might result, affording protection against radiation injury.

SUTTON and ROTBLAT [8] have shown in chemical reactions in aqueous systems exposed to 15-MeV electrons that the ratio G (radical reaction)/G (molecular reaction) falls when dose-rates of 10^9 r/s and above are used. This has been explained by the increased probability of track overlap and radical recombination. It is unlikely that the effects observed in this experiment could be ascribed to this phenomenon, since the dose-rates employed are far below the levels where this mechanism is likely to operate.

Though the sterility of the adults showed an increase with increase in dose-rate, no change in pattern of response, as seen in the eggs, was observed in the adults at high dose-rate. It is likely that the dose-rate needed to bring about a similar response in the adults must be of a higher order. No attempt could be made to test the validity of this assumption in view of the limitations of the present Co^{60} source.

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DISCUSSION

R.C. von BORSTEL: I think your results are extremely interesting. Is it possible that some of these dose-rate effects can be explained by changes in the cell cycle to radiation sensitivity during the period the embryos were irradiated? In Habrobracon, the cell cycle of the young embryo is only about 15 min long, and the sensitivity can vary over a twenty-fold range at least.

K.K. NAIR: Your suggestion is an excellent one. We have thought of this probability and it needs some ingenious experimentation to test its validity.

B.D. BAINES: With reference to Table I on the viability of eggs against dose-rate, is there a plateau at the minimum percentage viability shown at a dose-rate of 47 420 rad/h, or is this a point minimum? This is not clear, owing to the large difference between dose-rates of 3 520 and 81 550 rad/h on either side of the minimum shown. The exact nature of this minimum could be of great interest to irradiation plant designers.

K.K. NAIR: It is difficult to say whether or not this is a point minimum, as dose-rates immediately below 47 420 r and those above 81 550 r have not been listed for their effects. These studies are still in progress.

INFLUENCE DE L'IRRADIATION SUR LES ADULTES DE SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) ET LEURS DESCENDANTS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF IRRADIATION ON THE ADULTS OF SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) AND THEIR OFFSPRING. Co^{60} gamma rays bring about a significant reduction in expectation of life as from 5000 r in the case of males and 7000 r in that of females. All insects die within about twelve days after a dose of 8000 r and within four days after 100 000 r.

Study of the pattern of mortality as a function of time shows that, up to 6000 r, irradiation induces a progressive heterogeneity in the population, the more resistant individuals being isolated.

Examination of the survival curves shows that death is always deferred. It occurs suddenly after a latent phase, the duration of which varies, according to dose, between twelve days (5000 r) and one day (100 000 r).

Study of the variability coefficient and of the average survival time according to dose suggests that two sensitive mechanisms are destroyed by irradiation.

Radiosensitivity appears to increase with age.

Fertility is reduced by half as from 2000 r. A period of temporary sterility appears at 5000 and 6000 r, and complete sterility sets in at 15 000 r. Sterilization by X-rays is more effective at 150 than at 80 kV.

Egg production is much more radioresistant than fertility, eggs being laid even at 16 000 r.

Irradiation of parents induces a decrease in both fertility and weight of offspring and shows the latter's rate of development. These effects are transmitted to succeeding generations without any effort at selection on the part of the experimenter. The first two effects tend to disappear, but the decrease in the rate of development remains constant for at least ten generations.

It would seem possible to use radiation for disinfestation of foodstuffs.

INFLUENCE DE L'IRRADIATION SUR LES ADULTES DE SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) ET LEURS DESCENDANTS. Le rayonnement gamma de ^{60}Co réduit significativement la moyenne de vie des mâles à partir de 5000 r, et celle des femelles à partir de 7000 r. Tous les insectes meurent en une douzaine de jours à 8000 r, en quatre jours à 100 000 r.

Jusqu'à 6000 r, l'étude de la répartition de la mortalité dans le temps montre que l'irradiation fait apparaître progressivement une hétérogénéité dans la population, isolant des individus plus résistants.

L'examen des courbes de survie révèle que la mort est toujours différée. Elle apparaît brusquement après une phase de latence plus ou moins longue selon la dose, variant de douze jours (5000 r) à un jour (100 000 r).

L'étude du coefficient de variabilité et de la moyenne de survie en fonction de la dose suggère la présence de deux mécanismes sensibles détruits par l'irradiation.

La sensibilité au rayonnement semble augmenter avec l'âge.

Dès 2000 r, la fertilité se trouve réduite de moitié. A 5000 r et 6000 r, on observe une phase de stérilité temporaire. La stérilisation totale est acquise dès 15 000 r. La stérilisation par les rayons X est plus efficace à 150 kV qu'à 80 kV.

La fécondité est beaucoup plus résistante que la fertilité au rayonnement, puisque même à 16 000 r des œufs sont pondus.

L'irradiation des parents a pour conséquence de diminuer la fertilité et le poids des descendants, et d'augmenter leur durée de développement. Ces effets se transmettent d'une génération à l'autre sans que l'expérimentateur procède à aucune sélection. Alors que les deux premiers tendent à disparaître, l'augmentation de la durée du développement reste stable pendant au moins dix générations.

Il est possible d'envisager l'emploi des rayonnements pour la désinsectisation des denrées.

ДЕЙСТВИЕ ОБЛУЧЕНИЯ НА ВЗРОСЛЫХ ОСОБЕЙ SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) И НА ПОСЛЕДУЮЩИЕ ПОКОЛЕНИЯ. Облучение гамма-лучами Co^{60} значительно уменьшает среднюю продолжительность жизни мужских особей, начиная с дозы 5000 р, и женских особей - с дозы 7000 р. Все насекомые погибают в течение 12 дней при дозе 8000 р и четырех дней при дозе 100 000 р.

Исследование распределения смертности в зависимости от времени при дозе до 6000 р показывает, что облучение последовательно выявляет гетерогенность популяции, изолируя при этом более стойкие особи.

Изучение кривых выживания показывает, что наступление смерти постоянно замедлено. Она наступает внезапно после более или менее продолжительной латентной фазы в зависимости от дозы. Колебания составляют от 12 дней (5000 р) до одного дня (100 000 р).

Исследование коэффициента вариабильности и среднего срока выживания в зависимости от дозы предполагает наличие двух чувствительных механизмов, нарушаемых облучением.

Чувствительность к облучению, по-видимому, возрастает с возрастом. С 2000 р плодовитость сокращается вдвое. При 5000 и 6000 р наблюдается фаза временного бесплодия. Полная стерилизация достигается при дозе 15 000 р и выше. Стерилизация рентгеновскими лучами более эффективна при 150 кв, чем при 80 кв.

Способность к оплодотворению значительно более стойка, чем плодовитость, по отношению к облучению, потому что даже при 16 000 р продолжается откладывание яиц.

Облучение родителей ведет к сокращению плодовитости и веса последующих поколений и увеличению срока их развития. Эти последствия передаются от одного поколения другому, причем экспериментатор не ведет никакой селекции. В то время как два первых поколения имеют тенденцию к исчезновению, увеличение срока развития продолжает оставаться стабильным, по крайней мере, для десяти поколений.

Представляется возможным применение радиации для дезинсекции продуктов питания.

INFLUENCIA DE LA IRRADIACIÓN SOBRE LOS ADULTOS DEL SITOPHILUS SASAKII TAKAHASHI (CURCULIONIDAE) Y SUS DESCENDIENTES. La radiación gamma del ^{60}Co reduce significativamente la vida media de los machos a partir de 5000 r, y la de las hembras a partir de 7000. Todos los insectos mueren en doce días si la dosis es de 8000 r, y en cuatro días si es de 100 000.

Hasta 6000 r, el estudio de la distribución de la mortalidad en el tiempo muestra que la irradiación hace aparecer una heterogeneidad progresiva en la población, aislando los individuos más resistentes.

El examen de las curvas de supervivencia revela que la muerte siempre es diferida. Sobreviene bruscamente después de una fase de latencia más o menos prolongada según la dosis y que varía entre 12 días (5000 r) y un día (100 000 r).

El estudio del coeficiente de variabilidad y de la supervivencia media en función de la dosis sugiere la existencia de dos mecanismos sensibles que son destruidos por la irradiación.

La sensibilidad a las radiaciones parece aumentar con la edad.

A 2000 roentgens, la fertilidad queda ya reducida a la mitad. A 5000 y 6000 roentgens se observa una fase de esterilidad temporal. La esterilización total se alcanza a 15 000 roentgens. La esterilización por rayos X es más eficaz a 150 kV que a 80 kV.

La fecundidad es mucho mas resistente a las radiaciones que la fertilidad, pues incluso a 16 000 roentgens se observa la puesta de huevos.

La irradiación de los padres tiene por consecuencia la disminución de la fertilidad y del peso de los descendientes y el aumento de la duración de la fase de desarrollo. Estos efectos se transmiten de una generación a otra sin que el experimentador proceda a selección alguna. Mientras los dos primeros tienden a desaparecer, el aumento de la duración de la fase de desarrollo permanece estable durante diez generaciones, por lo menos.

Cabe prever el empleo de las radiaciones para la desinsectación de los artículos alimenticios.

Les insectes utilisés sont élevés à la température de 27, 5° (± 0, 2°) et 75% d'humidité relative (± 5%). Ils sont placés dans des boîtes à couvercle et fond grillagés et disposent en moyenne de 15 à 20 grains de blé par individu et par jour. Chaque boîte renferme au maximum 25 insectes pour la reproduction. La source d'irradiation est une bombe au ^{60}CO. Quel-

ques traitements ont été faits toutefois aux rayons X, avec différents voltages (80, 100, 120 et 150 kV) et un filtre d'aluminium de 0,5 mm.

A. ACTION SUR LA MORTALITÉ

1. Réduction de la durée moyenne de vie

Le tableau I donne les résultats bruts. L'analyse statistique par le test de Student prouve que l'influence du rayonnement γ se manifeste seulement à partir de 5000 r pour les mâles et entre 6000 r et 8000 r pour les femelles. Celles-ci sont donc plus résistantes à l'irradiation, bien que vivant moins longtemps que les mâles chez les témoins.

L'analyse de la répartition des pourcentages de mortalité en fonction du temps après l'irradiation (fig. 1) montre que dès 4000 r (dose non létale), la mortalité apparaît plus rapidement que chez les témoins (44^{e} jour). A 5000 r une phase primaire de mortalité se déclenche dès le 12^{e} jour, et elle s'accentue à 6000 r. Cette première phase ne dure que quelques jours, et elle est nettement séparée dans le temps de la phase de mortalité normale, qui n'apparaît, chez les irradiés comme chez les témoins, que le 65^{e} jour.

Les deux maxima (12^{e} et 86^{e} jours) ne correspondent pas à la sensibilité différente des deux sexes, et l'on doit conclure que l'irradiation entraîne l'apparition progressive d'une hétérogénéité dans la population d'insectes, isolant des individus plus résistants qui meurent ensuite au même rythme que les témoins.

2. Courbes de survie

L'examen de ces courbes (fig. 2) montre que même pour des doses fortement létales, la mortalité ne se déclenche qu'après une phase de latence au cours de laquelle les insectes continuent à se nourrir.

Ce fait a été signalé par d'autres auteurs [2, 8] et paraît constant si l'on irradie de jeunes imagos. Il met en évidence que les DL 50 calculées par de nombreux auteurs au bout d'un ou deux jours seulement ne rendent que partiellement compte de la radiosensibilité véritable des insectes.

Pour établir des comparaisons valables, il faudrait déterminer la dose réduisant de moitié la durée moyenne de vie: la DV 50. Elle serait voisine de 7000 r pour S. sasakii.

3. Etude de la variabilité

Le coefficient de variabilité (tableau II) augmente régulièrement avec la dose jusqu'à 6000 r et le test de Snedecor indique que la variance de chacune des séries irradiées est significativement différente de celle des témoins. Le rayonnement modifie donc la sensibilité des insectes aux variations incontrôlables du milieu.

Mais on doit remarquer que cet effet est bivalent: à 8000 r la variance diminue brusquement de près de 3000 fois. Puis de nouveau le coefficient de variabilité augmente régulièrement. Il paraît donc exister entre 6000 r et 8000 r un seuil que sépare deux types d'action différente du rayonnement [7].

TABLEAU I

MOYENNES DE SURVIE DE SITOPHILUS SASAKII
ÂGÉS DE MOINS DE TROIS SEMAINES
APRÈS DIFFÉRENTES DOSES DE RAYONS GAMMA

Doses (r)	Sexe	Nombres d'insectes	Moyennes de survie (j)
Témoins	Mâle	91	123,71
	Femelle	88	74,28
4 000	Mâle	103	124,75
	Femelle	83	71,24
5 000	Mâle	105	88,78
	Femelle	85	77,90
6 000	Mâle	98	43,78
	Femelle	103	67,48
8 000	Mâle	249	8,75
	Femelle	246	8,75
15 000	Mâle et Femelle	343	9,01
30 000	-	364	8,30
60 000	-	187	5,26
100 000	-	194	2,13

On doit remarquer par ailleurs que la moyenne de survie décroît rapidement de 4000 r à 8000 r (de plus de 10 fois). Puis de 8000 r à 15 000 r on obtient un palier. Enfin, au delà de 15 000 r, elle diminue à nouveau, mais plus lentement.

Cette analyse suggère l'intervention de deux mécanismes sensibles, dont l'un serait totalement détruit dès 8000 r, et l'autre atteint au delà de 15 000 r seulement.

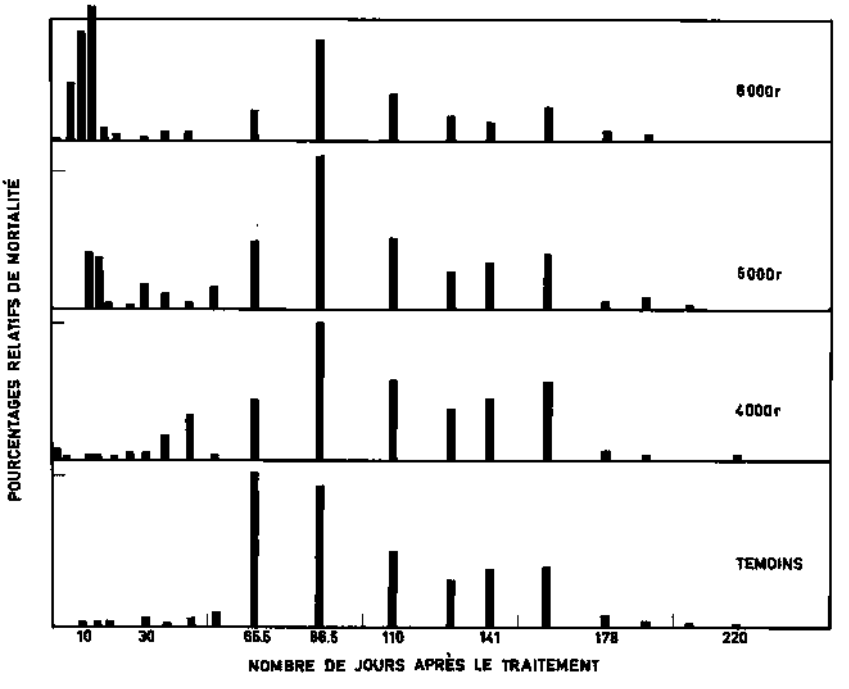

Figure 1

Répartition de la mortalité de Sitophilus sasakii dans le temps, après irradiation à 6000 r, 5000 r, 4000 r et chez les témoins.
En abscisses: nombre de jours après le traitement.
En ordonnées: pourcentages relatifs de mortalité.

4. Influence de l'âge sur la sensibilité au rayonnement

Les insectes irradiés étaient âgés de moins de trois semaines et leur radiosensibilité paraît très proche de celle des espèces voisines S. granarius et S. oryzae [2, 8], et également de celle de Anthonomus grandis [3]. Mais des expériences préliminaires montrent que la sensibilité au rayonnement augmente significativement avec l'âge dès que la différence dépasse un mois.

B. ACTION SUR LA REPRODUCTION

1. Etude de la fertilité

Nous entendons par fertilité le nombre d'adultes obtenus par femelle et par unité de temps.

- Le processus de reproduction est très sensible au rayonnement: alors qu'une femelle témoin produit en moyenne 249 descendants au cours de sa vie, une femelle irradiée à 2000 r n'en donne que 101, bien que cette dose n'ait aucune influence sur la longévité.

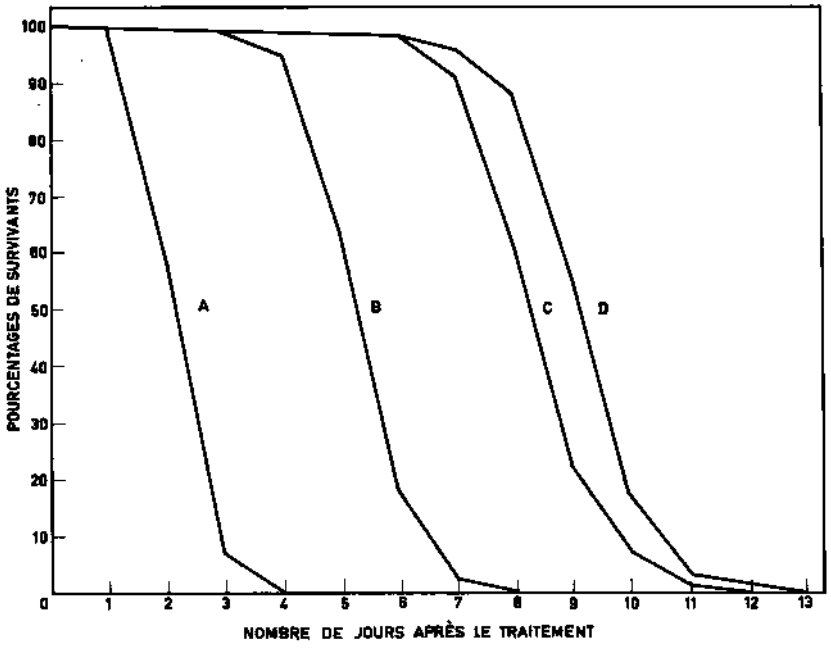

Figure 2

Courbes de mortalité de Sitophilus sasakii après irradiation à 15 000 r (D), 30 000 r (C), 60 000 r (B) et 100 000 r (A).
En abscisses: nombre de jours après le traitement.
En ordonnées: pourcentages de survivants.

- L'augmentation de la dose entraîne une inhibition supplémentaire de la fertilité. Ainsi, à 4000 r, elle est 25 fois plus faible chez les irradiés que chez les témoins pendant les 27 premiers jours. Au bout de deux mois, la stérilité est pratiquement acquise.
- A 5000 r apparaît une phase de stérilité temporaire du 7e au 12e jour après le traitement. Elle s'étend jusqu'au 19e jour à 6000 r. A 8000 r, 246 femelles n'ont donné que 18 descendants adultes.
- A 15 000 r la stérilisation est totale.
- Avec les rayons X, la stérilisation est plus efficace à 150 kV qu'à 80 kV: pour une dose de 8000 r, on obtient des descendants dans ce dernier cas, mais pas dans le premier.

2. Etude de la fécondité

La recherche des œufs pondus par les insectes irradiés [10] montre que le processus de ponte est beaucoup moins sensible au rayonnement. Ainsi, à 5000 r et 6000 r la ponte ne cesse pas pendant la phase de stérilité temporaire et des œufs sont encore pondus à 16 000 r. Ils n'éclosent pas, sans qu'on puisse dire à quel stade de développement s'est trouvé bloqué.

Ce phénomène paraît général. ERDMAN l'a fort bien démontré chez la guêpe Habrobracon [4] et chez Tribolium [5].

TABLEAU II

MODIFICATION DE LA VARIABILITÉ DE LA MOYENNE DE SURVIE EN FONCTION DE DIFFÉRENTES DOSES DE RAYONS GAMMA

Doses (r)	Témoins	4000	5000	6000	8000	10 000	15 000	30 000	60 000	100 000
Ecart type S	40, 19	45, 90	50, 59	53, 53	0, 98	0, 97	1, 39	1, 24	0, 87	0, 62
C = S/M	0, 40	0, 45	0, 59	0, 96	0, 11	0, 11	0, 15	0, 15	0, 17	0, 29

M = moyennes de survie C = Coefficient de variabilité

C. ACTION DU RAYONNEMENT GAMMA SUR LES DESCENDANTS

Nous avons constaté que, sans procéder à aucune sélection, l'irradiation des parents perturbe la physiologie des descendants pendant de nombreuses générations [9]. Ces effets sont résumés dans le tableau III.

TABLEAU III

INFLUENCE DU TRAITEMENT AUX RAYONS GAMMA DES PARENTS SUR LES DESCENDANTS

Caractères étudiés	Dose (r)	Moyennes au cours des générations successives					
		Parents	F_1	F_2	F_3	F_4	F_5
Poids (1/100 mg)	8000	-	-	125, 52	133, 00	-	143
	Témoins	-	-	144, 88	138, 18	-	144
Fertilité (nombre d'imagos par femelle et par jour)	5000	0, 08	2, 42	2, 62	3, 18	3, 07	-
	Témoins	4, 82	4, 37	4, 01	4, 19	3, 82	-
	8000	0, 01	0, 48	0, 67	1, 34	1, 36	-
	Témoins	3, 30	3, 60	3, 50	3, 92	3, 25	-
Durée de développement (j)	5000	-	32, 92	32, 99	32, 68	32, 78	32, 60
	Témoins	-	31, 48	31, 07	31, 35	31, 84	31, 79
	8000	-	34, 11	36, 41	35, 61	35, 59	36, 06
	Témoins	-	31, 14	31, 58	32, 81	32, 01	32, 03

1. Etude du poids

La diminution est très nette par rapport aux témoins en F_2 de la série irradiée à 8000 r ($p = 0, 001$ pour les mâles et $p = 0, 005$ pour les femelles). Elle tend à s'effacer en F_3 ($p = 0, 05$) et s'annule en F_4. Il se produit donc une régulation. Par ailleurs, la dispersion des poids est augmentée chez les descendants d'irradiés, à la fois par l'apparition d'individus de poids plus faible et plus élevé.

2. Etude de la fertilité

La diminution des taux de fertilité par femelle reste significativement inférieure à ceux des témoins jusqu'en F_4 où l'on a $p = 0, 05$ pour 5000 r et

p = 0,001 pour 8000 r. L'effet est statistiquement plus marqué dans cette dernière série.

Toutefois, on remarque que, là encore, une régulation se produit et la fertilité des descendants d'irradiés tend à reprendre la même valeur que chez les témoins.

3. Duré de développement

Elle est accrue dans les deux séries issues d'insectes irradiés et bien que la différence avec les témoins ne soit que de quelques jours, le test de Student indique qu'elle est très significative. Ici encore, l'effet de 8000 r est plus important; pour les cinq générations, on a $M_8 = 35,10$ j et $M_5 = 32,84$ j ($p = 0,001$).

Mais la variance intra-inter générations confirme qu'ici les moyennes restent stables au cours des cinq générations. Un calcul à la dixième génération indique encore la persistance de l'effet.

Pour l'instant, il n'est pas possible de donner une explication de ces phénomènes dont la nature paraît complexe. Des expériences sont en cours pour étudier dans le détail les modalités précises de la transmission.

D. POSSIBILITÉS D'EMPLOI DES RADIATIONS POUR LA DÉSINSECTISATION DES DENRÉES

D'après ces quelques résultats qui confirment ceux d'autres auteurs, il semble possible de détruire et stériliser facilement les charançons des grains. Une dose de 20 000 r paraît suffisante pour stériliser un quelconque insecte parasite des denrées entreposées [6].

Le fait que les stades immatures sont plus sensibles que les imagos aux radiations, que ces dernières ne laissent pas de résidus toxiques et permettent un traitement parfaitement homogène, milite en faveur de leur utilisation pour la désinsectisation des denrées. BROWNELL [1] et CORNWELL [2] ont déjà étudié des modalités d'application.

L'emploi des radiations comme insecticides doit cependant être envisagé comme une technique de complément plutôt qu'une technique de remplacement. En effet, en matière de lutte antiparasitaire, surtout en ce qui concerne les denrées entreposées, il est aussi important de prévenir que de stopper les infestations. Ainsi, l'emploi de poudre répulsive à long pouvoir d'action paraît-il présenter un intérêt majeur pour assurer la protection des denrées irradiées, dans tous les cas où le stockage lui-même (emballage ou ensilage) ne pourra garantir aucune nouvelle infestation.

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DISCUSSION

G. T. SCARASCIA: Do you think that the resistant specimens you found after irradiation are due to mutations or are they genotypes already present in the irradiated population?

P. NARDON: I have not studied the phenomenon in detail, but the resistance is not due to mutations, since these specimens are ones that have been irradiated - it is not the offspring. I think one must assume that some members of the population are more resistant than the rest. For that reason it is not unlikely that a certain degree of resistance to radiation can be acquired.

R. CAVALLORO: In irradiating the Sitophilus larvae, did you give single or fractionated doses?

P. NARDON: We administered single doses. Only adults were irradiated - not larvae. With an X-ray dose of 8000 r we got better sterilization at 150 kV than at 80 kV.

S. FUSEAU-BRAESCH: What exactly is meant by the "modifications" in sensitivity to uncontrollable environmental changes to which you refer in your paper?

P. NARDON: The insects were all irradiated at the same time and were then distributed into different boxes for statistical analysis. In view of the fact that the insects were all irradiated at the same time in an absolutely homogeneous part of the beam there can be no difference in the dose received. The variations observed in the insects in different boxes are therefore of the same type as those observed in the controls, which also show variations in fertility, weight, etc. In the case of the irradiated insects the variations are more marked and in my opinion they can only be due to uncontrollable environmental factors, such as temperature differences during handling of the insects caused by opening and closing of doors, small variations in relative humidity, etc.

S. FUZEAU-BRAESCH: This assumption of altered sensitivity to environmental factors is, of course, only a hypothesis, but personally I am not sure that it is satisfactory. The various biological disturbances, such as those caused by irradiation, do not normally have large-scale repercussions from the statistical point of view, but they do broaden the distribution spectra and this is something we encounter in many biological phenomena. I also wanted to ask you whether you obtain normal insect progeny or have you found abnormal specimens?

P. NARDON: We have found a few abnormalities of the legs and of the genital organs when using X-rays, but never with gamma radiation.

S. FUZEAU-BRAESCH: With regard to what is perhaps the most important part of your paper, namely the study of the offspring of your insects, I think such work has been neglected in the past and these modifications,

which are not strictly speaking chromosomal though they are found in several successive generations, should really be studied further. With regard to the loss of weight and fertility, the general reduction in vitality, do you think there is then a general regulatory process such that the vitality may exceed that of the controls? I think it was David in Lyons who got additional regulatory functions, but if I remember correctly he was not using radiation but certain toxic substances.

P. NARDON: The development period stays increased until the tenth generation, but I did not go beyond that. The effect on the weight disappears after the fifth generation, and we obtained a number of giant specimens. The fertility is also regulated, but I did not obtain any heightened fertility. Of course, I did not test the fertility during the whole lifetime because these grain weevils live for about a year and it would have required very complicated experimental arrangements. I only tested the fertility for a period of two weeks.

S. FUZEAU-BRAESCH: I think these modifications, which you have observed over ten generations, are highly interesting and they draw our attention to the general problem of the long-term phylogenetic effects of radiation such as might be caused by atomic explosions, fall-out, etc.

R. CAVALLORO: I believe that Cornwell, Crook, Bull and others reported that with Curculionidae, insects of the same family as those you refer to, a dose of 20 000 r gave only 6% sterilization. You stated that with a dose of 15 000 r you obtained total sterilization. Can you explain this difference?

P. NARDON: If I am not mistaken, Cornwell published some more recent work, in 1959, in which he got exactly the same results as those I quoted. I think these results also agree with those reported by several other authors, including, for example, Dr. von Borstel.

EFFECTS OF GAMMA RADIATION ON THREE SPECIES OF PHILIPPINE INSECT PESTS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF GAMMA RADIATION ON THREE SPECIES OF PHILIPPINE INSECT PESTS. A study was conducted on the effects of gamma radiation from a Co^{60} source on three agricultural insect pests, namely (1) the tomato lady beetle, Epilachna philippinensis Dieke, a pest of tomato and eggplant; (2) the rice weevil, Sitophilus oryzae L.; and (3) the rust-red flour beetle, Tribolium castaneum Herbst. The last two species are the most common and most destructive pests of corn and other stored grains and grain products in the Philippines.

Eggs, larvae, pupae and adults of E. philippinensis on tomato and adults of S. oryzae and T. castaneum on corn were used.

Four doses were used on Epilachna, namely 1, 5, 10 and 20 kr. In the other two species, five doses were used, 10, 20, 40, 60 and 80 kr.

At 1 kr, the proportion of unhatched eggs in Epilachna was about 10%. There was no proportionate increase in mortality at 5 kr, but at 10 kr the mortality increased to more than three times that at 5 kr. Mortality at 20 kr was about 90%. The threshold dose seems to lie between 5 and 10 kr. The embryo of eggs that did not hatch failed to continue development. At 1 kr, over 20% of the larvae were killed, although some of these succeeded in pupating before they died. Most of the larvae attained the adult stage. All the larvae that survived the treatments above 1 kr, some for as long as four weeks, died without attaining the pupal stage. The pupae were more resistant than the larvae and the adults. Complete mortality was not attained even with 20 kr. Some adults which emerged in the 5-kr and 10-kr lots had abnormally developed wings and/or legs. In the adults, the 10 and 20 kr doses had an adverse effect, killing about 75% of the individuals subjected to the lower, and all subjected to the higher, dose three weeks after irradiation. No significant difference in susceptibility between the sexes was noted.

About 40% of the S. oryzae in the lots treated with 60 and 80 kr were killed one week after irradiation, but only about 10% were killed with 20 kr and about 20% with 40 kr. The mortality with 40, 60 and 80 kr increased to about 75% two weeks after the start of the test. The mortality with 10 kr corresponding to this period was less than 10% and that with 20 kr was about 25%. The threshold dose seems to lie between 20 and 40 kr.

The mortality trend in the T. castaneum was similar to that in the S. oryzae, except that the threshold dose seems to lie at about 40 kr.

EFFETS DES RAYONS GAMMA SUR TROIS ESPÈCES D'INSECTES NUISIBLES DES PHILIPPINES. On a étudié les effets des rayons gamma provenant d'une source au ^{60}Co sur trois espèces d'insectes nuisibles pour l'agriculture: 1. l'épilachne de la tomate (Epilachna philippinensis Dieke) parasite de la tomate et de l'aubergine; 2. le charençon du riz (Sitophilus oryzae L.); 3. le ténébrion roux (Tribolium castaneum Herbst). Les deux dernières espèces sont les plus répandues et les plus nuisibles des parasites du maïs et autres céréales engrangées ainsi que des denrées à base de céréales aux Philippines.

On a étudié les effets des œufs, des larves, des pupes et des adultes de E. philippinensis sur la tomate et les effets des adultes de S. oryzae et T. castaneum sur le maïs.

Pour l'épilachne, on a utilisé quatre doses: 1, 5, 10 et 20 kr. Pour les deux autres espèces, on a employé cinq doses: 10, 20, 40, 60 et 80 kr.

Pour 1 kr, le pourcentage des œufs non éclos d'épilachne était d'environ 10%. On n'a pas constaté une augmentation proportionnelle de la mortalité pour 5 kr; mais pour 10 kr, la mortalité était plus de trois fois supérieure à ce qu'elle était pour 5 kr. Avec 20 kr, elle était d'environ 90%. La dose seuil semble se situer entre 5 et 10 kr. L'embryon des œufs non éclos ne continuait pas à se développer. Pour 1 kr, plus de 20% des larves étaient anéanties, bien que certaines d'entre elles eussent réussi à se métamorphoser en pupes avant de mourir. La plupart des larves atteignaient le stade adulte. Toutes les larves qui ont survécu à des irradiations

de plus de 1 kr, certaines pendant des périodes allant jusqu'à quatre semaines, sont mortes sans avoir atteint le stade de la pupe. Les pupes se sont révélées plus résistantes que les larves et que les adultes. On n'est pas parvenu à une mortalité totale, même avec 20 kr. Certains adultes apparus dans des lots irradiés à 5 et 10 kr avaient des ailes et/ou des pattes anormalement développées. Chez les adultes, les doses de 10 et 20 kr avaient des effets nocifs puisqu'elles anéantissaient, trois semaines après l'irradiation, 75% environ des individus ayant été soumis à la première dose et tous ceux qui avaient reçu la seconde. On n'a pas constaté de différence notable de sensibilité entre les sexes.

Dans les lots ayant reçu 60 et 80 kr, près de 40% de S. oryzae mouraient une semaine après l'irradiation, contre 10% seulement pour 20 kr et environ 20% pour 40 kr. Avec 40, 60 et 80 kr, la mortalité atteignait 75% deux semaines après le début de l'expérience. Pour 10 kr, la mortalité au terme de la même période était inférieure à 10% et pour 20 kr elle était d'environ 25%. La dose de seuil semble se situer entre 20 et 40 kr.

La mortalité de T. castaneum était comparable à celle de S. oryzae si ce n'est que la dose seuil semble se situer aux environs de 40 kr.

ДЕЙСТВИЕ ГАММА-ИЗЛУЧЕНИЯ НА ТРИ РАЗНОВИДНОСТИ НАСЕКОМЫХ-ВРЕДИТЕЛЕЙ НА ФИЛИППИНАХ. Изучалось действие гамма-излучения от источника Co^{60} на трех сельскохозяйственных насекомых-вредителях, а именно: 1) томатного жука Epilachna philippinensis Dieke, вредителя томатов и баклажанов; 2) рисового амбарного долгоносика Sitophilus oryza (L) и 3) красного мучного жука Tribolium castaneum (Herbst.). Последние две разновидности являются наиболее распространенными и опасными вредителями зерна и других хранящихся зерновых продуктов на Филиппинах.

При проведении опытов на томатах использовались яйца, личинки, куколки и взрослые особи E. philippinensis, а на зерне - взрослые особи S. oryza и T. castaneum.

Четыре дозы были применены для Epilachna, а именно: 1, 5, 10 и 20 кр. При обработке других двух разновидностей использовались пять доз: 10, 20, 40, 60 и 80 кр.

При дозе в 1 кр количество невылупившихся из яиц личинок у Epilachna составляло приблизительно 10%. При увеличении дозы до 5 кр не наблюдалось пропорционального увеличения смертности, но при дозе в 10 кр смертность возросла более чем в три раза, по сравнению со смертностью при дозе в 5 кр. Смертность при дозе в 20 кр составляла приблизительно 90%. Пороговая доза находится, по-видимому, в диапазоне между 5 и 10 кр. Зародыши яиц, из которых не вывелись личинки, перестали развиваться. При дозе 1 кр более 20% личинок погибло, причем некоторые из них окуклились, прежде чем погибнуть. Большинство личинок развилось во взрослые особи. Все личинки, которые вышли после облучения дозой свыше 1 кр, а некоторые из них жили в течение 4 недель, погибли, не достигнув стадии окукливания. Куколки оказались более устойчивыми, чем личинки и взрослые особи. Стопроцентная смертность не была достигнута даже при облучении 20 кр. У некоторых взрослых особей, подвергшихся облучению дозами в 5 и 10 кр, наблюдалось неправильное развитие крыльев и/или конечностей. У взрослых особей, подвергшихся облучению дозами 10 и 20 кр, наблюдался противоположный эффект, выражавшийся в гибели через три недели после облучения приблизительно 75% особей, облученных дозой 10 кр, и всех особей, облученных дозой 20 кр.

Около 40% S. oryza в партиях, получивших 60 и 80 кр, погибли через неделю после облучения. Однако только около 10% всех особей погибли после облучения 20 кр и около 20% особей - после 40 кр. Смертность после облучения 40, 60 и 80 кр увеличилась приблизительно до 75% через две недели после начала эксперимента. Смертность при дозе в 10 кр, соответствующая этому периоду, составляла менее 10%, а при дозе в 20 кр около 25%. Пороговая доза, по-видимому, лежит в диапазоне между 20 и 40 кр.

Тенденция к гибели у T. castaneum была подобной тенденции у S. oryza, за исключением того факта, что пороговая доза лежит, по-видимому, около 40 кр.

EFECTOS DE LAS RADIACIONES GAMMA SOBRE TRES PLAGAS INSECTILES DE LAS FILIPINAS. Se han estudiado los efectos de las radiaciones gamma de una fuente de ^{60}Co sobre tres insectos nocivos para la agricultura: 1) el escarabajo del tomate Epilachna philippinensis Dieke, que ataca al tomate y a la berenjena; 2) el gorgojo del arroz Sitophilus oryzae L., y 3) el escarabajo de la harina Tribolium castaneum Herbst. Las dos últimas especies son las plagas más corrientes y destructivas del maíz y de otros granos y derivados de granos ensilados en las Filipinas.

Los autores emplearon los huevos, larvas, ninfas e imagos del E. philippinensis en tomates, e imagos de S. oryzae y T. castaneum en el maíz.

Con el Epilachna se ensayaron cuatro dosis de 1, 5, 10 y 20 kr. Con las otras dos especies se ensayaron cinco dosis de 10, 20, 40, 60 y 80 kr.

Tras una dosis de 1 kr, el porcentaje de huevos hueros de Epilachna fue aproximadamente del 10%. La dosis de 5 kr no provocó un aumento proporcional de la mortalidad, pero con 10 kr la letalidad fue el triple que la correspondiente a 5 kr. Con una dosis de 20 kr, el índice de mortalidad alcanzó aproximadamente el 90%. Al parecer, la dosis umbral está comprendida entre 5 y 10 kr. En los huevos sin abrir, el embrión dejó de desarrollarse. Con 1 kr, murieron más del 20% de las larvas, aunque algunas de ellas llegaron a formar ninfas. La mayor parte de las larvas alcanzaron el estado adulto. Las larvas que sobrevinieron a dosis de más de 1 kr, algunas durante hasta cuatro semanas, murieron sin llegar a formar ninfas. Las ninfas resultaron más resistentes que las larvas y los insectos adultos, pues la mortalidad completa no se alcanzó ni siquiera con dosis de 20 kr. Algunos de los adultos que surgieron de los grupos que habían sido expuestos a dosis de 5 y 10 kr tenían alas y/o patas anormales. Las dosis de 10 kr matan aproximadamente al 75% de los adultos a las tres semanas de haberlos irradiado, y 20 kr bastan para matar a todos al cabo del mismo tiempo. No se registraron diferencias importantes entre los sexos en cuanto a la sensibilidad.

Aproximadamente el 40% de los S. oryzae que habían recibido dosis de 60 y 80 kr murieron una semana después de la irradiación; en cambio, las dosis de 20 kr sólo mataron a un 10% y las de 40 kr al 20%. La mortalidad con dosis de 40, 60 y 80 kr aumentó hasta un 75% a las dos semanas de haber comenzado el ensayo. La mortalidad con 10 kr correspondiente a este período resultó inferior al 10%, y con 20 kr alcanzó aproximadamente el 25%. Al parecer, la dosis umbral parece encontrarse entre 20 y 40 kr.

La mortalidad en el caso del T. castaneum resultó similar que para el S. oryzae, con la salvedad de que la dosis umbral parece encontrarse hacia los 40 kr.

The use of gamma radiation for the control of various insect pests has been the subject of various investigations abroad in a number of countries. In the Philippines, no work has been done in this field of research.

Of major concern in this country is the control of agricultural insect pests, which have been undermining the economy of the country for decades. Insects are responsible for millions of pesos' worth of damage to Philippine agriculture. A case in point is that of the pests of stored grains and copra. Maize, which is the staple food in some regions of the country, could not be stored for more than two to three months without extensive damage by insect pests. As a result, this foodstuff cannot be stocked to provide a continuous supply of the commodity throughout the year. Another example is the case of the copra beetle, Necrobia rufipes De G. One of the major causes of the poor quality of Philippine copra in the world market is the damage inflicted by this insect on the stored product. Various insecticides have been found very effective against these pests, but as the products are processed or manufactured into food the use of poisons is ruled out, for obvious reasons.

The need for basic information on the effects of radiation on Philippine insect pests of economic importance has long been felt. With the reported success, at least in some instances, of the utilization of gamma radiation in the field of pest control abroad, considerable interest has been shown in this field of investigation by local entomologists and food processors. A programme of studies along this line has been initiated at the Philippine Atomic Research Centre. Radiosensitivity studies of some of our important insect pests are now being undertaken. It is expected that with the completion of the Philippine research reactor and the Co^{60} facility of the Philippine Atomic Research Centre of the Philippine Atomic Energy Commission in a few months from now, research along this line will be stepped up.

The present study is confined to the gross effects of gamma radiation on the insects. A more detailed study has recently been initiated. Part of the material used in the present investigation will be studied for microscopic effects of radiation.

MATERIALS AND METHODS

In the present investigation, a study was conducted on the effects of gamma radiation on three species of agricultural insect pests, namely, the tomato lady-beetle, Epilachna philippinensis Dieke, the rice-weevil, Sitophilus oryzae L. and the rust-red flour beetle, Tribolium castaneum Herbst. The first species is the most common pest of tomato and the other two are the most common and most destructive pests of stored cereals.

The radiation source used in this investigation was a Co^{60} teletherapy machine giving 39 r/min at a distance of 70.5 cm from the source at the time of the tests. The source had a strength of 1996 c on 18 September 1958, when it started operation.

The irradiation dose rates used on E. philippinensis were 1, 5, 10 and 20 kr. In S. oryzae and T. castaneum, the dose rates were 10, 20, 40, 60 and 80 kr. These rates were determined after a preliminary test. The specimens for irradiation were placed in short plastic vials with loose caps. The insects were transferred to rearing containers with feed soon after treatment.

Eggs, fifth-instar larvae, pupae and adults of E. philippinensis varying in age from one to two days were used in the tests. In order to provide a steady supply of the various metamorphic stages, the insect was mass-reared on tomato plants grown on plots in a screenhouse and in wire-screen cages in the laboratory.

The mortality of the irradiated eggs and the larvae from the hatched eggs in all treatments were determined. In addition, the percentage of living larvae that attained the adult stage, the fertility of the eggs laid by these adults and any abnormality in the larvae and adults were also recorded. In the irradiated larvae, the percentage mortality of the larvae and the pupae and other pertinent observations were taken. Similar observations were conducted on the irradiated pupae and adults, but the mortality of the adults in both cases was taken four weeks from the date of irradiation.

Approximately one-week-old adults of S. oryzae and T. castaneum were irradiated at 10, 20, 40, 60 and 80 kr. The percentage mortality of the adults was taken 30 d after irradiation. Observations were continued beyond 30 d to determine the reproductive potential of the irradiated insects.

RESULTS AND DISCUSSION

Epilachna philippinensis Dieke

Egg. The results obtained with the irradiation of the eggs are shown in Table I. The lethal effect of the gamma radiation on the eggs is clearly evident from the percentage of eggs that failed to hatch. Microscopic exami-

TABLE I

EFFECTS OF GAMMA RADIATION ON EGGS OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality (%)		Living larvae that attained adult stage (%)	Remarks
	Eggs	Larvae		
0 (Control)	3.1	4.8	95.2	All adults normal. Fertility of eggs laid about 93%.
1	10.2	37.3	70.2	All adults apparently normal. Fertility of eggs laid about 95%.
5	25.7	60.7	39.3	About 6% of adults had abnormal legs and/ or wings. Fertility of eggs laid about 92%.
10	81.8	92.1	5.3	About 10% of adults had abnormal legs and/ or wings. Adults incapable of reproduction. No eggs laid.
20	90.5	96.8	2.4	About 25% of adults had abnormal legs and/ or wings. Adults incapable of reproduction. No eggs laid.

nation of the unhatched eggs showed that the embryo failed to continue development. The mortality is dose-dependent – the higher the dose the more lethal the effect. This effect was carried to the larval and subsequent developmental stages. Beyond 10 kr, however, the increase in mortality was not commensurate with the increase in dose. An investigation on the lethal and sterilizing effects of gamma radiation on insects infesting cereal commodities by some workers showed that beyond 6 000 and up to 50 000 rep there was only a slight reduction in the time required to obtain a 100% kill [4]. The adults which developed from the eggs irradiated with 1 kr were all apparently normal and laid eggs with a fertility of about 95%. In the lot treated with 5 kr, some of the adults that developed had abnormal legs and/or wings, but the eggs they laid were fertile. More adults with abnormal appendages appeared in the lot treated with 10 kr. All the adults which emerged from this treatment were incapable of reproduction and no eggs were laid. The same was observed in the eggs irradiated with 20 kr. In addition, about one-fourth of the adults that emerged from this lot had abnormal appendages. The existence of a threshold dose between 5 and 10 kr is evident. The eggs of this species seem more resistant than those of T. castaneum, which require a dose of only 11 100 rad for the development of adults from irradiated eggs to be completely prevented [6].

Fifth-instar larvae. Table II shows the effect of the various dose rates of radiation on the fifth-instar larvae. The larvae were much more sensitive to radiation than the eggs. At 1 kr, about one-fourth of the larvae were killed, the rest attaining the pupal stage. Only 85.7% of the pupae reached the adult stage but about 35% of the adults had abnormal wings and/or legs. The eggs laid, however, had about the same fertility as those of the control. The higher dose-rates (5, 10 and 20 kr) had very deleterious effects on the larvae. About one week after irradiation with 5 kr, all the larvae became inactive and stopped feeding. The larvae turned yellow to orange and all died in about a month without attaining the pupal stage. The larvae treated with 10 kr exhibited practically identical conditions. At 20 kr the larvae stopped feeding and became inactive about 24 h after irradiation. All turned yellow and died within two weeks after irradiation without attaining the pupal stage. There was a protracted delay in the pupation of the larvae. Some of the larvae treated with 5 and 10 kr lived for about a month without attaining the pupal stage. It is quite possible, as in the case of Drosophila, that the delay in pupation is caused by hormonal disturbance in the larva [3]. The larvae of this species are much more susceptible to gamma radiation than those of the rust-red flour beetle, T. castaneum, as reported by CROOK [6].

Pupa. (Table III) The pupae were more resistant to gamma radiation than the larvae. At 1 kr, about 5% of the pupae were killed and about 15% of the adults that emerged died within four weeks after irradiation. The remaining adults died within six weeks. The fertility of the eggs laid by the adults was about 90%. All the adults which emerged from the lots treated with 5 and 10 kr died within four weeks after irradiation. About 10% of the adults which emerged in the lot treated with 5 kr had abnormal appendages. The fertility of the eggs laid was only about 40%. In the pupae treated with 10 kr, only about 5% of the adults had abnormal appendages. All the adults died within four weeks after irradiation without laying eggs. About one-fourth of the adults that emerged in the lot treated with 20 kr had abnormal

TABLE II

EFFECTS OF GAMMA RADIATION ON FIFTH-INSTAR LARVAE OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality (%)	Pupae that attained adult stage (%)	Remarks
0 (Control)	0	100	All adults normal. Fertility of eggs laid about 95%.
1	22.2	85.7	About 34.5% of adults had abnormal wings and/or legs. Fertility of eggs laid about 91%.
5	100	-	Larvae became inactive and stopped feeding about one week after irradiation; turned bright yellow or orange. All died in about a month from irradiation without attaining pupal stage.
10	100	-	As above.
20	100	-	Larvae stopped feeding and became inactive about 24 h after irradiation. All turned yellow and died within two weeks from date of irradiation without attaining pupal stage.

appendages. All adults died within two weeks after irradiation without laying eggs.

Adult. (Table IV) The adults treated with 1 kr were active and appeared unaffected by the radiation. Like those in the control lot, they all died within seven weeks after treatment and the fertility of the eggs laid was about 94%. In the lot treated with 5 kr, the fertility of the eggs laid was about 72% and about 63% of the larvae attained the adult stage. At 10 kr some adults became inactive after two or three days and all the adults died within five weeks after irradiation. The fertility of the eggs laid was about 60% and approximately 50% of the larvae attained the adult stage. The adults treated with 20 kr became inactive soon after irradiation and died within three weeks. The fertility of the eggs laid was only about 30%. Of the total larvae that emerged, close to 15% attained the adult stage.

The data obtained on this species indicate differences in the susceptibility of the various metamorphic forms of the insect to gamma radiation. The eggs showed marked resistance to radiation, about 10% of those treated with 20 kr attaining the adult stage. The fifth-instar larva was the most susceptible of the various metamorphic forms of the insect tested. There was no significant difference in susceptibility between sexes. In stored-grain insects, it has been reported that graded resistance within a species was exhibited, increasing with the development stage from egg to adult [8]. On the basis of the data obtained in this investigation it appears that E. philippinensis is more susceptible to gamma radiation than S. oryzae and T. castaneum. The data obtained on the last two species seems to agree with those found by other workers [2, 5, 6, 8, 11].

Sitophilus oryza and Tribolium castaneum

The results on the irradiation of the stored grain insects are shown in Table V. Of the two species studied, S. oryzae appeared more susceptible to gamma radiation than T. castaneum. The mortality in the lot treated with 10 kr two weeks after irradiation was 17.3% in the case of S. oryzae and 5.8% in the case of T. castaneum. The figures corresponding to these species in the 20-kr treatment were 46.4 and 16.7%. At 40 kr the mortality in S. oryzae was 86.3% as against 64.1% in the T. castaneum. In S. oryzae the increase in mortality from 40 to 60 kr was only 0.8%, and from 60 to 80 kr 9.6%. In T. castaneum the corresponding figures were 9.3 and 3.3%. The mortality at 80 kr was 96.7% in the former species and 76.7% in the latter. In both species there was only a slight increase in mortality from dose to dose above 20 kr compared with the dose-to-dose increase below 20 kr.

Thirty days after irradiation, about one-third of the S. oryzae and about one-ninth of the T. castaneum were killed. The 20-Kr treatment killed about 70.7% of the former and about 38.6% of the latter. The corresponding figures in the 40 kr treatment were 100 and 92.3% respectively. At 60 kr, all the T. castaneum were killed within 30 d. The existence of a threshold dose, particularly in the case of T. castaneum, is very evident.

It has been reported [7] that a dose of 8 400 r prevented T. confusum from reproducing. The possibility of grain-weevils providing a biological dosimeter for radiation disinfestation of grains had been considered. The results of other investigators [4], have, however, shown that this method

TABLE III

EFFECTS OF GAMMA RADIATION ON PUPAE OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Pupae that attained adult stage (%)	Mortality of adults four weeks after irradiation (%)	Remarks
0 (Control)	100	0.3	All adults normal. Fertility of eggs laid about 96%. About 15% of adults died within six weeks from start of tests.
1	94.7	15.3	All adults apparently normal. All adults died within six weeks from time of irradiation. Fertility of eggs laid about 90%.
5	61.5	100	About 10% of adults with abnormal wings and/or legs. All adults died within four weeks from time of irradiation. Fertility of eggs laid about 40%.
10	43.8	100	About 5% of adults with abnormal wings and/or legs. All died in four weeks after irradiation. No eggs laid.
20	16.7	100	About 25% of adults with abnormal wings and/or legs. All died within two weeks after irradiation. No eggs laid.

TABLE IV

EFFECTS OF GAMMA RADIATION ON ADULTS OF EPILACHNA PHILIPPINENSIS DIEKE

Treatment (kr)	Mortality four weeks after irradiation (%)	Remarks
0 (Control)	1.3	All adults active; about 25% died within seven weeks from start of tests. Fertility of eggs laid about 91%.
1	11.6	All adults active like those in control; all died within seven weeks after irradiation. Fertility of eggs laid about 94%.
5	60.4	Adults active; all died within seven weeks after irradiation. Fertility of eggs laid about 72%. About 63% of larvae attained the adult stage.
10	96.7	Some adults became inactive 2 to 3 d after irradiation. All adults died within five weeks from time of irradiation. Fertility of eggs laid about 60%. About 50% of larvae attained adult stage.
20	100	Some adults became inactive soon after irradiation. All adults died within three weeks from time of irradiation. Fertility of eggs laid about 30%. About 15% of larvae attained adult stage.

TABLE V

MEAN MORTALITY OF SITOPHILUS AND TRIBOLIUM ADULTS AT VARIOUS DOSE-RATES 30 d AFTER IRRADIATION (%)

Species	Control	At dose-rates (kr): 10	20	40	60	80
Sitophilus oryza	1.8	32.3	70.7	100	100	100
Tribolium castaneum	2.6	11.2	38.6	92.3	100	100

holds little promise. A more detailed study on the effects of gamma radiation on all species of stored product was recently started at the Philippine Atomic Research Centre.

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DISCUSSION

C. P. PAPADOPOULOU: Did you observe any recoveries during this period of one month?

G.B. VIADO: No, there was no instance in which the insect completely recovered.

P. NARDON: With regard to the phenomena you observed following the irradiation of Epilachna larvae in the fifth stage you referred to the possibility of a hormonal disturbance, and I think that it is indeed a possibility which one can hardly ignore in many cases; the grain-weevil, as I mentioned earlier, experiences changes in growth period, fertility and weight, and such disturbances are also obtained with insecticides, which indicates that there may be some action on the neuro-secretion system.

G.B. VIADO: Yes; we have not made a detailed study of the hormonal aspects but it does look as though there is something of that nature.

PRELIMINARY STUDIES ON IRRADIATION OF SOME COMMON STORED-GRAIN INSECTS IN PAKISTAN

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Abstract — Résumé — Аннотация — Resumen

PRELIMINARY STUDIES ON IRRADIATION OF SOME COMMON STORED-GRAIN INSECTS IN PAKISTAN. The response of some major insect pests of stored grains to various radiation dosages from a Co^{60} source made available through the courtesy of the Pakistan Atomic Energy Commission has been studied. A dosage of 20 000 r produced 100% mortality of the adults of Rhizopertha dominica and Tribolium castaneum within a period of 11 d. A lower dose (10 000 r) was, however, effective in 23 d. The adults of Sitophilus granarius evinced comparatively high susceptibility and when they were exposed to 10 000 r, total mortality was attained in 7 d. The larvae of Trogoderma granarium, however, proved to be the most resistant; they were not responsive to the lower dosages, and 100% control was attained only with 25 000 r in 26 d. The maximum dosage of 250 000 r gave an instant kill in all these cases, but apart from being too high it also adversely affected the viability of the grains. Since, however, their nutritive value remained unaffected, this treatment might be of use where immediate disinfestation of non-seeding edible grains was desired. Eggs of Bruchus quadrumaculatus failed to hatch when exposed to 2500 r. Studies on other stages and dosages are in progress.

ÉTUDES PRÉLIMINAIRES SUR L'IRRADIATION DE CERTAINES ESPÈCES COMMUNES D'INSECTES NUISIBLES AUX CÉRÉALES ENGRANGÉES AU PAKISTAN. On a étudié la réponse à différentes doses de rayonnements de quelques espèces d'insectes particulièrement nuisibles aux céréales engrangées; on a utilisé à cet effet une source au ^{60}Co fournie par la Commission pakistanaise de l'énergie atomique. Une dose de 20 000 r a provoqué en l'espace de 11 j une mortalité de 100% chez les adultes de la Rhizopertha dominica et du Tribolium castaneum. Une dose de 10 000 r a donné le même résultat en 23 j. Les adultes du Sitophilus granarius ont montré une sensibilité beaucoup plus élevée; une dose de 10 000 r a entraîné une mortalité de 100% en 7 j. Ce sont les larves de la Trogoderma granarium qui se sont révélées les plus résistantes; elles ont été insensibles aux doses de faible intensité et la mortalité de 100% n'a été obtenue qu'avec 25 000 r, en 26 j. Une dose maximum de 250 000 r a entraîné une mort instantanée dans tous les cas; mais, outre le fait qu'elle est trop élevée, cette dose nuit à la conservation des céréales. Toutefois, la valeur nutritive des céréales ne s'en trouvant pas affectée, on pourrait appliquer des doses de cette intensité lorsqu'on veut rapidement désinsectiser des grains comestibles qui ne sont pas destinés à la semence. Des œufs de Bruchus quadrumaculatus exposés à une dose de 2500 r ne sont pas parvenus à éclosion. On poursuit les études sur des insectes à d'autres stades de leur développement et pour des doses différentes.

ПРЕДВАРИТЕЛЬНОЕ ИЗУЧЕНИЕ РЕЗУЛЬТАТОВ ОБЛУЧЕНИЯ НЕКОТОРЫХ ОБЫЧНЫХ АМБАРНЫХ НАСЕКОМЫХ В ПАКИСТАНЕ. Были изучены ответные реакции некоторых основных амбарных насекомых-вредителей на облучение различными дозами от источника с C^{60}, любезно предоставленного Пакистанской комиссией по атомной энергии. Доза 20 000 р вызвала 100-процентную смертность взрослых особей Rhizopertha dominica и Tribolium castaneum в течение 11 суток. Доза 10 000 р оказалась эффективной только через 23 суток. Взрослые особи Sitophilus granarius проявляли относительно большую чувствительность, и при облучении их 10 000 р полная смертность наступала через 7 суток. Однако личинки Trogoderma granarium оказались наиболее стойкими; на более низкие дозы они вовсе не реагировали, а 100-процентная смертность была достигнута только через 26 суток после облучения 25 000 р. Максимальная доза 250 000 р приводила к мгновенной смерти во всех случаях, но эта доза слишком высока, и приводит к нарушению прорастаемости семян. Однако ввиду того, что питательные свойства не страдают, эта доза может быть использована там, где желательно немедленное истребление насекомых в непредназначенных для посева продовольственных зерновых. Bruchus quadrumaculatus не вылупливается при облучении дозой в 2500 р. В настоящее время продолжается изучение других стадий и дозировок.

ESTUDIOS PRELIMINARES SOBRE LOS EFECTOS DE LA IRRADIACIÓN EN ALGUNAS DE LAS PLAGAS INSECTILES DE LOS SILOS MÁS COMUNES EN EL PAKISTAN. El autor ha estudiado los efectos que en algunas de las principales plagas insectiles de los silos ejercen diversas dosis de irradiación aplicadas con una fuente de ^{60}Co que puso a su disposición la Comisión de Energía Atómica del Pakistán. Una dosis de 20 000 r causó al cabo de 11 d una mortalidad del 100% entre los Rhizopertha dominica y Tribolium castaneum adultos. Una dosis de 10 000 r produjo los mismos resultados al cabo de 23 d. El Sitophilus granarius adulto acusó una sensibilidad relativamente mucho más elevada, pues con una dosis de 10 000 r se obtuvo una mortalidad del 100% en sólo 7 d. Las larvas de Trogoderma granarium demostraron ser las más resistentes; con dosis inferiores no acusaron efecto alguno y su exterminio total exigió 25 000 r y 26 d. Una dosis máxima de 250 000 r produjo inmediatamente efectos letales en todos los insectos, pero aparte de ser demasiado elevada resultó perjudicial para la vitalidad de los granos. Ahora bien, como no altera su poder nutritivo, se puede emplear cuando se quiera lograr la desinsectación inmediata de granos comestibles que no sean para la siembra. Los huevecillos de Bruchus quadrumaculatus dejan de desarrollarse cuando sufren una irradiación de 2500 r. El autor está estudiando los efectos de otras dosis en otras fases de la metamorfosis.

INTRODUCTION

Of the several factors limiting crop production, insect pests occupy a place of great economic significance. They ravage the crops not only from the seedling to the growing stage but in addition continue to steal their share from the field to the storage bins. All the world over, insects constitute one of the most important hazards to the safe storage of grains. They have been estimated to take a toll of at least 5% of the world production of cereal grains. A survey conducted by the Food and Agriculture Organization in 1947 indicated that, in 29 countries, of the total loss of cereal grains of 12.5 million tons about 50% was caused by insect pests alone [1]. For a predominantly agricultural country like Pakistan, the problem of the safe and clean storage of grain and grain products was a problem of its very economic viability. The tropical and sub-tropical climate of the country is highly favourable to the growth and multiplication of stored-grain insects. Of the total of about 50 insect species recorded as infesting grains, about 10 are reckoned as pests of major importance that account for at least 3-5% of the country's much-needed staple grains. On a conservative estimate, the annual insect damage in State storage alone is of the order of one million rupees.

The conventional methods of controlling these insects have so far varied between the age-old sun treatment and the modern insecticidal or fumigation methods. Fumigation, though by far the most effective method of grain disinfestation, has its own serious limitations. The efficiency and efficacy of application are governed by the physical factors of distribution and penetration and are subject to human error as well as to several indeterminable factors; then there are hazards of application as well as the toxicity of residues left behind. The development of immunity or resistance amongst the insects through persistent application of chemical insecticides is yet another incentive to devise and develop a better method of grain disinfestation which will take care of the basic control problem without leaving any toxic or poisonous residue.

With the discovery of radioisotopes and the phenomenon of radiation, there have recently been a great many studies on their exploitation and utilization towards the solution of many entomological problems. Apart from the use of radioisotopes as tagging or labelling material for the determination of

various ecological and physiological complexes and manifestations of insect life, studies on achieving population control through direct irradiation have acquired great impetus during the recent past. Though the first known application of ionizing radiation for the control of the cigarette beetle (Lasioderma serricorne) dates back as far as 1912 [3], it has only been since the early fifties that the problem has been studied in greater and more practical detail. Considerable work on the radiation disinfestation of cosmopolitan stored-grain insects has since been carried out in different countries, particularly the United Kingdom, Australia, the United States and the Soviet Union. The effect of radiation on the various life stages, the doses required, and the sequence of their effect on grains have been under constant investigation. HASSETT and JENKINS irradiated six species of stored-product insects with X-rays [5]. They concluded that heavy infestations could be quickly killed with a dose of 65 kr and that lesser doses might prevent reproduction and control higher infestation levels. CORNWELL and BULL, while working on adults of Sitophilus granarius and Sitophilus oryzae, derived a very high dose, above 300 kr, for killing these insects in less than 24 hours [2]. Lower doses of 15 kr and 25 kr gave complete control within about a week and no mortality was observed to occur below 2 kr. A dose of 0.5 Mrad was found by HORNE and BROWNELL to cause reproductive sterilization and death within 24 hours [4]. About 0.1 Mrad induced a radiation lethargy such that subsequent feeding and damage were minimized, leading finally to death within a week. Lower doses, in the range of 15-20 kr, also caused sterilization, but total mortality was delayed.

The effects of gamma irradiation of Rhizopertha dominica, Cadra (Ephestia) cautella, Plodia interpunctella and Lasioderma serricorne carried out by PENDLEBURY et al. [6] showed that the emergence of adult Rhizopertha dominica was not affected by doses of up to 20 000 rad. The emergence of Cadra cautella males was reduced by 50% at 28 400 rad and that of females at 11 600 rad. In Plodia interpunctella a 60-70% reduction in emergence was brought about by 36 000 rad, there being no difference in susceptibility between the sexes. Emergence in Lasioderma serricorne was unaffected by doses of up to 36 000 rad. Pupae and adults of Rhizopertha dominica were equally susceptible to gamma radiation.

These studies have in large measure demonstrated the economic as well as the technical feasibility of irradiation as a practical method of grain-insect control. In order, therefore, to determine the specific response of the local species and strains to the various radiation dosages and to establish its optimum rate, preliminary studies on the irradiation control of some of the important grain pests have been conducted in Pakistan. On the basis of these laboratory studies, which are being continued, it is proposed to launch a project on the large-scale control of stored-grain pests. A brief résumé of the material studied, methods adopted and results obtained is presented in the following lines.

METHOD AND MATERIAL

There are two potential methods of insect control through irradiation, indirect and direct. The indirect method consists in releasing sterile males

into the infested hold or habitat. On account of certain biological and ecological limitations, however, success so far has been confined to a limited number of species; the eradication of the screw-worm (Callitroga hominivorax) in the south-eastern United States is the only outstanding example of the success of the sterile-male technique up till now. The present series of experiments relate to direct irradiation, i.e. exposure of test insects to gamma radiation from a Co^{60} source in a "Gamma-Cell-220". This is a commercial irradiator designed for laboratory trials by Atomic Energy of Canada limited. The source is heavily shielded and is almost completely foolproof and safe for the applicators and research workers. The field in the gamma cell is provided by a Co^{60} source set up in the form of a squirrel cage. The chamber provided therein holds samples for irradiation when lowered into the source cage. The whole process of introducing the material for irradiation and removing it afterwards is designed to operate in an automatic, "push-button" manner.

The insects selected for the present study were Sitophilus granarius, Rhizopertha dominica, Tribolium castaneum, Trogoderma granarium (khapra) and Callosobruchus subinnotatus, which have been found to be the major pests of rice, wheat, wheat products and pulses during storage. Stock cultures of these insects were reared and maintained under their respective optimum conditions of temperature and humidity. 50 adults, each 1-2 weeks old, of Sitophilus granarius, Rhizopertha dominica and Tribolium castaneum, and third-instar larvae of Trogoderma granarium were placed in 3-in polyethylene tubes. Each tube contained 10 g of grain (gram in the case of Callosobruchus and wheat for the others) and was covered with muslin. All the batches of adults and larvae were then subjected to gamma irradiation from the Co^{60} source described above. Several doses ranging between 2.5 and 250 kr at a dose-rate of 8.0×10^5 r per hour were tried, each dose being given three replications. After irradiation, the larvae and the adults were returned to the controlled optimum conditions of temperature and humidity. Controls were also separately kept for each species and each dose.

RESULTS AND CONCLUSIONS

From the results obtained (Tables I-IV) it will be seen that while in all the four cases of Rhizopertha dominica, Tribolium castaneum, Sitophilus granarius and Trogoderma granarium 100% mortality immediately after exposure was obtained with the maximum radiation dosage of 250 kr, the minimum lethal dosages varied in each case. The minimum dose of 10 kr produced a 100% kill in 23 d amongst adults of both Rhizopertha dominica and Tribolium castaneum. Although the complete kill was very much delayed, the insect ceased to evince feeding activity after irradiation, so that further damage was precluded. Increased dosages of 20, 50, 80, 100, 120 and 150 kr against Rhizopertha dominica were effective in producing a total kill in 11, 9, 8, 6, 5 and 3 d respectively. The rate of control therefore more or less directly varied with the dosage rate. Similarly, in the case of Tribolium castaneum the higher dosages of 20, 50, 80, 100, 120 and 150 kr were effective in 11, 9, 7, 6, 5 and 3 d respectively. The responses of

TABLE I

EFFECT OF GAMMA RADIATION ON RHIZOPERTHA DOMINICA AND TRIBOLIUM CASTANEUM
(50 insects per treatment)

Insect and stage	Dose (kr)	Mortality (mean of 3 replications) (%) after (d)											
		0 (Immediately after exposure)	2	3	5	6	7	8	9	10	11	14	23
Rhizopertha dominica (adults)	10	-	-	-	-	-	70	-	90	-	-	-	100
	20	-	-	30	-	50	60	-	90	-	100	-	-
	50	-	-	40	40	-	50	60	100	-	-	-	-
	80	-	-	50	70	-	80	100	-	-	-	-	-
	100	-	50	80	90	100	-	-	-	-	-	-	-
	120	-	80	-	100	-	-	-	-	-	-	-	-
	150	-	80	100	-	-	-	-	-	-	-	-	-
	250	100	-	-	-	-	-	-	-	-	-	-	-
	Nil (control)	-	-	-	-	-	-	-	-	-	-	-	-
Tribolium castaneum (adults)	10	-	-	-	70	-	-	80	-	-	90	-	100
	20	-	-	30	50	60	-	90	-	100	-	-	-
	50	-	-	40	40	-	50	60	100	-	-	-	-
	80	-	50	70	-	80	100	-	-	-	-	-	-
	100	-	50	80	90	100	-	-	-	-	-	-	-
	120	-	80	100	-	-	-	-	-	-	-	-	-
	150	-	80	100	-	-	-	-	-	-	-	-	-
	250	100	-	-	-	-	-	-	-	-	-	-	-
	Nil (control)	-	-	-	-	-	-	-	-	-	-	-	-

TABLE II

EFFECT OF GAMMA RADIATION ON SITOPHILUS GRANARIUS (ADULTS)
(50 insects per treatment)

Dose (kr)	Mortality (mean of 3 replications) (%) after (d)				
	0 (Immediately after exposure)	2	3	5	7
10	-	25	55	75	100
20	-	50	90	90	100
50	-	50	80	100	-
100	100	-	-	-	-
Nil (control)	-	-	-	-	-

Rhizopertha dominica and Tribolium castaneum adults to the various dosages of gamma rays were, therefore, identical.

Sitophilus granarius, however, proved to be the most susceptible when exposed to 10 kr and 20 kr; a 100% kill was attained within 7 d. The next higher dose of 50 kr was effective in giving total control in 5 d. Larvae of Trogoderma granarium turned out to be most resistant of all the test insects. Lower doses of the order of 2.5 kr and 5 kr did not produce any adverse effect. Radiation dosages of 10, 15 and 25 kr also proved practically ineffective, because total kill was obtained only after 30, 28 and 26 d respectively. Complete mortality immediately after irradiation was obtainable only with the maximum dosage of 250 kr. However, 3-d-old khapra eggs exposed to 10 kr gave only 50% hatching. The emerged larvae also died within 3 d, and there was no emergence at all with increased dosages of 15 and 25 kr. The 1-d-old eggs of Callosobruchus subinnotatus when exposed to 3 and 5 kr failed to give any emergence, while at 2.5 kr the life cycle was extended to 48 d from 39 d for the control. Similarly the 12-d-old grubs did not moult and died after 18, 16, 16 and 10 d when exposed to 5, 10, 15 and 20 kr.

The behaviour of the adults of Rhizopertha dominica, Tribolium castaneum and Sitophilus granarius to various radiation dosages as discussed above indicated that they were capable of being controlled within the dose range 10 – 50 kr. The maximum dosage of 250 kr, though giving instant kill, was considered high and thus unsuitable for commercial operation, as the cost involved would not be economical.

The observations on khapra larvae, which have so far defied economical control, are considered inconclusive. Further studies on khapra and other insects are being continued.

TABLE III

EFFECT OF GAMMA RADIATION ON TROGODERMA GRANARIUM

Stage	No. of larvae or eggs	Dose (kr)	Mortality (mean of 3 replications) (%) after (d)																			
			0 (Immediately after exposure)	6	8	9	10	11	12	14	16	18	20	22	23	24	26	28	30	32	34	36	40
Third-instar larvae	50	5	-	12	-	-	-	-	20	-	30	-	44	50	54	-	62	-	75	-	85	95	100
		10	-	20	-	-	40	-	-	50	-	-	55	60	-	-	75	95	100	-	-	-	-
		15	-	20	-	-	37	-	50	-	60	-	75	-	-	80	90	100	-	-	-	-	-
		20	-	25	-	-	45	-	-	65	-	72	85	-	-	88	100	-	-	-	-	-	-
		25	-	35	40	-	55	-	60	-	77	-	85	86	-	90	100	-	-	-	-	-	-
		250	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Nil (control)[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Stage	No. of eggs	Dose (kr)	Emergence (%)
3-d-old eggs	100	10	50
		15	Nil
		25	Nil
		Nil (control)[b]	87

[a] Pupation began after the 18th day of the experiment.
[b] But no larva survived more than 3 d.

TABLE IV

EFFECTS OF GAMMA RADIATION ON EGGS AND GRUBS OF CALLOSOBRUCHUS SUBINNOTATUS
(100 eggs or grubs per treatment)

Stage	Dose (kr)	Results (mean of 3 replications)
24-h-old eggs	2	Emergence delayed by 9 d
	3	No emergence
	5	No emergence
	Nil (control)	95% usual hatching took place after 8 d
Second-stage grubs (12 d old)	5	No moulting and all died on the 18th day
	10	No moulting and all died on the 16th day
	15	No moulting and all died on the 16th day
	20	No moulting and all died on the 10th day
	Nil(control)	Normal moulting took place after 6-8 d.

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DISCUSSION

E.E. TURTLE: You stated that Cornwell and Bull had reported a very high dose for grain disinfestation. I think the dose they indicated was one

which would have an immediate killing effect. In countries where the grain may be stored for some time an immediate kill is not necessary and consequently a lower dose can be used.

H. HUQUE: That is correct. Sometimes we have to export rice to other Far Eastern countries, such as Thailand, and in that case we need to know the dose which will kill the insects within a certain period of time.

STUDIES ON THE EFFECTS OF GAMMA RADIATION ON THE DIFFERENT DEVELOPMENTAL STAGES OF THE KHAPRA BEETLE, TROGODERMA GRANARIUM EVERTS

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Abstract — Résumé — Аннотация — Resumen

STUDIES ON THE EFFECTS OF GAMMA RADIATION ON THE DIFFERENT DEVELOPMENTAL STAGES OF THE KHAPRA BEETLE, TROGODERMA GRANARIUM EVERTS. Different stages in the development of the Khapra beetle were exposed to various doses of gamma radiation and their effects were recorded. It was observed that the females were more susceptible to gamma radiation than the males. A dose of 6000 rad induced sterility in the females, whereas the males required 16 000 rad for complete sterility. The reasons for such differences are discussed.

In addition the influence of post-irradiation temperature on radiation damage has been investigated. The response of the larvae to gamma radiation appeared to be considerably influenced by temperature following irradiation. Thus the LD50 (8 d) at temperatures of 30°C, 34°C and 38°C was 59 980, 26 360 and 24 100 rad respectively.

Evidence is presented to show that, within certain limits, the stage at which the radiation damage manifests itself is largely dependent on the ambient temperature. In the irradiated larvae maintained at 30°C, pupation was arrested above a dose of 8000 rad. In those maintained at 34°C and 38°C doses above 10 000 and 12 000 rad respectively were required to bring about the same effect. It is well known that a reduction in ambient temperature prolongs the larval stage. This is reflected in this study by the fact that at low temperature mortality occurred in the larval stage itself, whereas at higher temperature it was seen in the pupal stage.

EFFETS DES RAYONS GAMMA AUX DIVERS STADES DU DÉVELOPPEMENT DU DERMESTIDÉ KHAPRA, TROGODERMA GRANARIUM (EVERTS). Le dermestidé Khapra a été exposé, à divers stades de son développement, à des doses variables de rayons gamma dont on a noté les effets. On a observé que les femelles étaient plus sensibles que les mâles aux rayons gamma. Une dose de 6000 rad a provoqué la stérilité chez les femelles, alors qu'il en fallait 16 000 pour provoquer la stérilité complète chez les mâles. Les auteurs discutent les raisons de ces différences.

En outre, les auteurs ont étudié l'influence de la température après irradiation sur les dommages radioinduits. La sensibilité des larves aux rayons gamma semble être fortement influencée par la température après l'irradiation. Par exemple, la DL_{50} (8 j) à des températures de 30°C, 34°C et 38°C était respectivement de 59 980, 26 360 et 24 100 rad.

Les auteurs indiquent des faits qui portent à croire que, dans certaines limites, le stade auquel les dommages radioinduits se manifestent dépend dans une large mesure de la température ambiante. Chez les larves irradiées maintenues à 30°C, la pupation était arrêtée pour une dose supérieure à 8000 rad. Chez les larves maintenues à 34 et 38°C, il fallait, pour obtenir le même effet, des doses de 10 000 et 12 000 rad respectivement. Il est bien connu que la diminution de la température ambiante prolonge le stade larvaire. Cette prolongation se traduit, dans cette étude, par le fait qu'aux basses températures la mortalité est apparue au stade larvaire, tandis qu'aux températures plus élevées elle a été constatée pendant la pupation.

ИССЛЕДОВАНИЕ ВЛИЯНИЯ ГАММА-ОБЛУЧЕНИЯ НА РАЗЛИЧНЫЕ ФАЗЫ РАЗВИТИЯ ЖУКОВ КАПРА, TROGODERMA GRANARIUM (ЭВЕРТСА). Жуки Капра на разных фазах развития подвергались гамма-облучению в различных дозах с регистрацией последствий облучения. Было замечено, что женские особи более чувствительны к гамма-облучению, чем мужские. Доза 6000 рад приводит к стерильности у женской особи, тогда как для полной стерилизации мужской особи требуется 16 000 рад. Обсуждаются причины такого различия.

Кроме того, исследовалось влияние на течение радиационного повреждения температуры окружающей среды после облучения. Реакция личинок на гамма-облучение, как кажется, в значительной степени зависит от температуры, при которой они содержатся после облучения. Таким образом, LD_{80} (8дней) при температурах 30°C, 34°C и 38°C соответственно равнялась 59 980, 26 360 и 24 100 рад.

Приводятся доказательства того, что с некоторыми ограничениями стадия, на которой проявляется радиационное повреждение, в значительной степени зависит от температуры окружающей среды. Окукливание личинок, содержащихся при температуре 30°C, прекращалось при дозе свыше 8000 рад. Для получения такого же эффекта прекращения окукливания личинок, содержащихся при температурах 34°C и 38°C, требовались дозы сверх 10 000 и 12 000 рад соответственно. Хорошо известно, что снижение температуры окружающей среды продлевает стадию личинки. Это подтверждается в данном исследовании тем, что при низкой температуре смертность наблюдается в самой стадии личинки, тогда как при более высокой температуре она наблюдается в стадии окукливания.

ESTUDIO DE LOS EFECTOS DE LAS RADIACIONES GAMMA SOBRE LAS DIVERSAS FASES DE DESARROLLO DEL COLEÓPTERO TROGODERMA GRANARIUM (EVERTS). Los autores expusieron T. granarium en diversas fases de desarrollo a dosis variadas de radiaciones gamma y registraron los efectos observados. Las hembras se mostraron más sensibles a las radiaciones gamma que los machos, pues quedaron estériles con una dosis de 6000 rad mientras que los machos precisaron 16 000 rad para llegar a la esterilidad completa. La memoria discute los motivos de esta diferencia.

Además, se ha investigado la influencia que ejerce sobre las radiolesiones la temperatura a que se mantiene al insecto después de la irradiación. Se encontró que la reacción de las larvas a las radiaciones gamma varía bastante con dicha temperatura. Las DL_{50} (8 d) a temperaturas de 30°C, 34°C y 38°C fueron 59 980, 26 360 y 24 100 rad respectivamente.

La memoria demuestra que, dentro de ciertos límites, la fase en que se manifiestan los daños radioinducidos depende en gran parte de la temperatura ambiente. Manteniendo a 30°C las larvas irradiadas, la formación de ninfas se interrumpió con dosis superiores a 8000 rad. Para lograr el mismo efecto con larvas mantenidas a 34°C y 38°C se precisaron, respectivamente, dosis superiores a 10 000 y a 12 000 rad. Como se sabe, al reducir la temperatura ambiente se prolonga la duración del estado larval. El presente estudio refleja esto en el hecho de que la mortalidad a temperaturas bajas se produjo en el propio estado larval, mientras que a temperaturas más elevadas se produjo en las ninfas.

INTRODUCTION

For the efficient utilization of radiation in the disinfestation of grain it is imperative that the minimum dose suggested for commercial treatment must be effective against all the known insect pests in the grain. Extensive studies carried out by CORNWELL, P.B. and MORRIS, J.A. [6] on the susceptibility of Sitophilus granarius and S. oryzae to gamma radiation have shown that a dose of 16 krad was sufficient for complete control of these insects in the grain. MARTIN, V.J. et al. [11] have indicated that changes in population densities will not affect the susceptibility of grain weevils to sterilizing doses of gamma radiation and that it is unlikely to reduce the efficacy of 16 krad for the control of this insect. PENDLEBURRY, J.B. and BANHAM, E.J. [12] studied the influence of culture temperature in modifying the radiation damage in the grain weevil and concluded that, though changes in both the pre- and the post-irradiation temperatures modify radiation response at certain dose levels, the efficacy of the recommended dose will not be reduced by the "fluctuating and variable temperatures such as are found in commercial practice".

It is well known that changes in environmental factors, such as low temperature and scarcity of food, can induce "diapause" or quiescence in the larvae of the Khapra beetle, Trogoderma granarium [3]. It is therefore

reasonable to expect that under such conditions of suspended animation the response of the larvae to gamma radiation will be different from what it would be when they are irradiated during a period of high physiological activity. The present study was undertaken with a view to assessing the role of "diapause" in modifying the radiation damage in the larvae, and also the susceptibility of the various stages of development of the Khapra beetle to different doses of gamma radiation.

MATERIAL AND METHODS

The developmental stages of the Khapra beetle used in these studies were taken from cultures maintained in the laboratory at 38 ± 1°C. Irradiation was carried out in a cobalt-60 source at a dose-rate of 1.5×10^5 rad/h.

In order to evaluate the sterilizing dose for both the sexes, male and female pupae were collected from the culture over a period of four days at one-day intervals and pooled to give random distribution of all the age groups. Requisite numbers were then taken and irradiated with doses ranging from 5 to 18 krad. After irradiation the male and female pupae were kept separately for adult emergence along with a set of controls. The emerged adults were then paired as follows:- (a) Irradiated male × normal female, (b) Normal male × irradiated female, (c) Irradiated male × irradiated female and (d) Normal male × normal female. For each mating combination three replications of fifteen pairs were used. The insects thus paired were kept in specimen tubes (8 cm × 2.5 cm) along with crushed wheat to serve both as food and as a medium for oviposition. The food was sieved for the eggs at the end of four days and on alternate days thereafter till all the females died. Observations on the viability of the eggs were made five days after oviposition.

For studies on the lethal effects of radiation, two-day-old eggs were irradiated with doses ranging from 2 to 6 krad. In each replication 50 eggs were taken and each dose treatment was replicated three times. The viability of the irradiated and control eggs was recorded at the end of five days. Wherever hatching occurred the larvae were provided with food and allowed to complete development.

Freshly hatched larvae were irradiated with doses ranging from 5 to 16 krad. For each dose three replications of 100 larvae each were taken. After irradiation they were released over crushed wheat in Petri dishes kept at 38°C. Mortality as well as pupation rates were recorded till all the larvae had either pupated or died. In addition, larvae in the late instar and prepupal stage were also irradiated with doses ranging from 40 to 150 krad. Mortality counts were taken at the end of 48 h and the data were subjected to "probit" analysis for the estimation of LD 50.

In order to determine whether post-irradiation temperature would modify radiation response, larvae collected at random from the culture maintained at 34 ± 1°C and which consisted of instars after the third, were irradiated with doses ranging from 5 to 18 krad. After irradiation they were separated into three groups. Of these, one was transferred to a temperature of 30°C, while the second and the third were kept at 34 and 38°C respectively. The data obtained on mortality at the end of the eighth day were subjected to "probit" analysis for the calculation of LD50.

RESULTS

It is seen from Table I that the eggs when exposed to a dose of 4 krad and above failed to hatch. At 2 krad the viability, pupation and adult emergence were 19.3%, 7% and 5.4% respectively, while in the control the corresponding figures were 88.6%, 74.7% and 65.4% respectively.

TABLE I

EFFECT OF GAMMA RADIATION ON THE EGGS OF TROGODERMA GRANARIUM EVERTS

Dose (krad)	Viability (%)	Pupation (%)	Adult emergence (%)
0 (Control)	88.6	74.7	65.4
2	19.3	7.0	5.4
4	0	0	0
5	0	0	0
6	0	0	0

The results obtained on the response of the freshly hatched larvae to gamma radiation are presented in Table II. These data show that 100% mortality was obtained at all dose levels of 10 krad and above on the 25th day after irradiation. At a dose level of 5 krad, about 14% underwent pupation, but adult emergence was completely inhibited. At all the other dose levels the larvae did not enter into the next instar and died in the first instar itself.

Table III gives the effects of radiation of the last instar and prepupal stages. It can be seen that the LD50 at the end of 48 h is 101 900 rad, with fiducial limits at 95% confidence level of 101 600 and 102 100 rad.

When adults which emerged from irradiated pupae were paired as indicated earlier it was observed that fecundity decreased with increasing dose (Fig. 2). When a mating combination was exposed to a dose of 6 krad all the eggs were found to be sterile. On the other hand when only the male in this combination was given a dose of 6 krad viability was observed to be 26.5%. A further reduction in the viability occurred when the dose was raised from 6 krad, and 100% sterility was obtained when the males received a dose of 16 krad (Fig. 1). These data indicate that the females can be sterilized by a dose of 6 krad while the corresponding sterilizing dose for the males is 16 krad.

The effects of temperature on the susceptibility of the larvae to gamma radiation are recorded in Table IV. It is evident from the data that the radiation susceptibility was more pronounced at 34 and 38 than at 30°C. The

TABLE II

RESPONSE OF FRESHLY HATCHED LARVAE OF TROGODERMA GRANARIUM EVERTS TO DIFFERENT DOSES OF GAMMA RADIATION

Dose	Mortality (%) after									Pupation
(krad)	9	15	25	36	47	53	66	70	73 d	(%)
0 (Control)	5.0	5.0	10.0	10.0	10.0	-	-	-	-	90.0
5	11.0	25.0	29.0	48.0	61.0	62.0	72.0	79.0	86.0	14.0
6	29.0	46.0	55.0	79.0	96.0	100.0				0.0
8	63.0	87.0	94.0	100.0						0.0
10	85.0	97.0	100.0							0.0
12	98.0	98.0	100.0							0.0
14	90.0	97.0	100.0							0.0
16	90.0	96.0	100.0							

TABLE III

RELATIVE SUSCEPTIBILITY OF THE LATE-INSTAR LARVAE AND PREPUPAL STAGE OF TROGODERMA GRANARIUM EVERTS TO GAMMA RADIATION

Heterogeneity	Regression equation	LD50 (rad)	Fiducial limits of LD50 with 95% confidence
χ^2 (6) = 5.76	Y = 4.771 x - 18.89	101 900	102 100 101 600

Y = probit kill; x = log dose; LD50 = Radiation dose calculated to give 50% kill. The data were not found to be heterogeneous at P = 0.05.

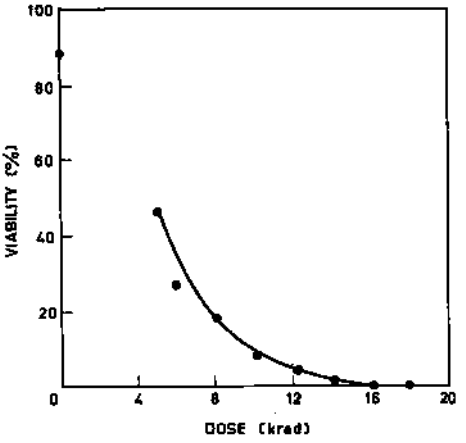

Fig. 1

Viability of eggs laid by normal female paired with male exposed to different doses of gamma radiation

LD50 values calculated from the probit analysis of mortality data were 59 980 rad at 30°C and 26 360 rad and 24 100 rad at 34 and 38°C respectively. The mortality response at doses of 14, 16 and 18 krad was independent of the post-irradiation temperature (Figs. 4, 6 and 8). However, at lower dose levels the influence of temperature on the mortality response was evident. It can also be noted that the pupation response was not only influenced by the radiation dose but also by the post-irradiation temperature treatment. At the three temperatures of 30°, 34° and 38°C pupation occurred at dose levels up to 8, 10 and 12 krad respectively. However, it was found that adults failed to eclose from the pupae formed at dose levels of 8 krad and above, irrespective of the post-irradiation temperature.

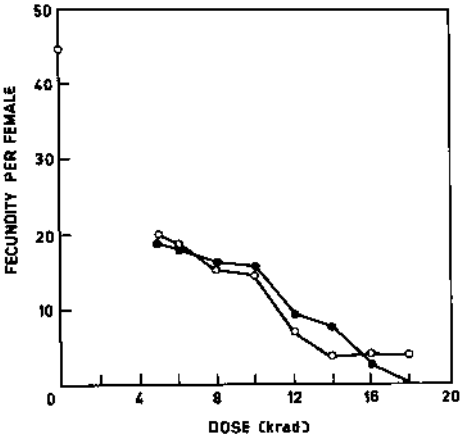

Fig. 2

Average number of eggs per irradiated female when paired with a normal or an irradiated male

● Irradiated ♂ × irradiated ♀
○ Normal ♂ × irradiated ♀

TABLE IV

RELATIVE SUSCEPTIBILITY OF THE LARVAE OF TROGODERMA GRANARIUM EVERTS TO GAMMA RADIATION IN RELATION TO POST-IRRADIATION TEMPERATURE

Post-irradiation temperature (°C)	Heterogeneity	Regression equation	LD50 (rad)	Fiducial limits of LD50 with 95% confidence
30	χ^2 (6) = 10.72	Y = 1.511 x - 2.224	59 980	63 360 56 780
34	χ^2 (6) = 7.3	Y = 1.953 x - 3.632	26 360	27 200 25 550
38	χ^2 (6) = 8.65	Y = 1.793 x - 2.875	24 100	25 060 23 120

Y = Probit kill; x = Log dose; LD50 = Radiation dose calculated to give 50% kill.
In none of the cases were the data found to be heterogeneous at P = 0.05.

DISCUSSION

It is evident from these studies that the females of the Khapra beetle are more susceptible to radiation than the males. A similar observation was made by CARNEY, G. C. [5] on the same species. Various factors

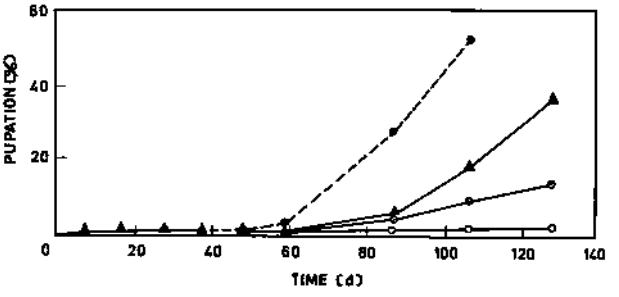

Fig. 3

Effect of post-irradiation temperature of 30°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
● Control

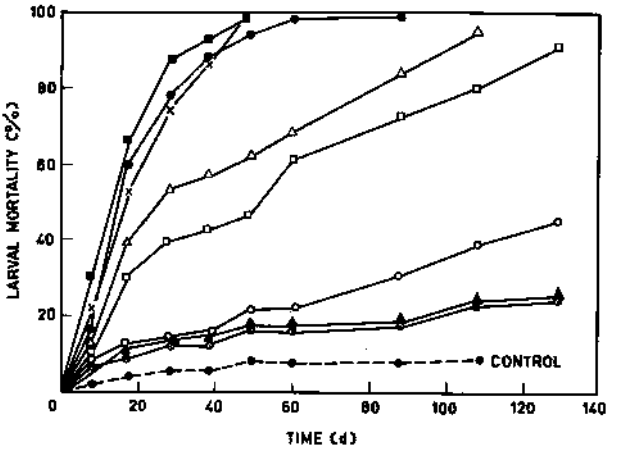

Fig. 4

Effect of post-irradiation temperature of 30°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

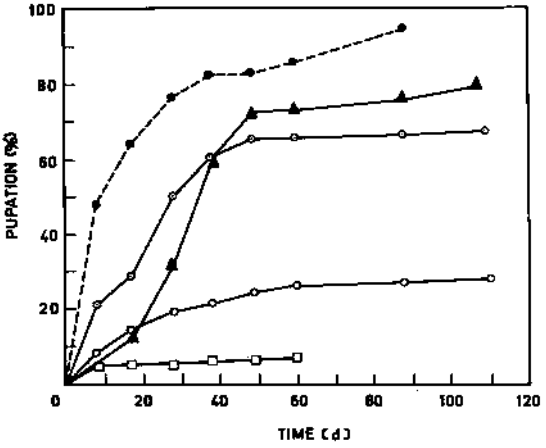

Fig. 5

Effect of post-irradiation temperature of 34°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
—●—●— Control

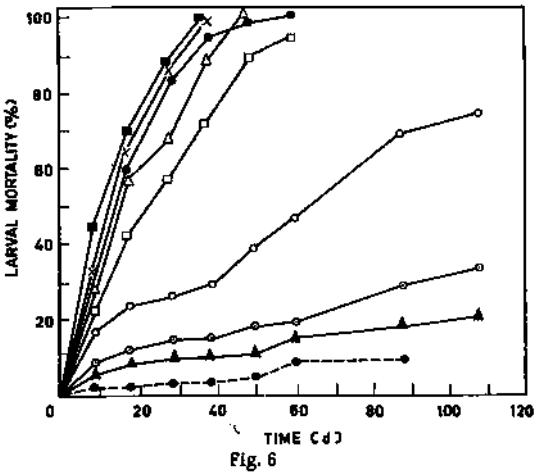

Fig. 6

Effect of post-irradiation temperature of 34°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

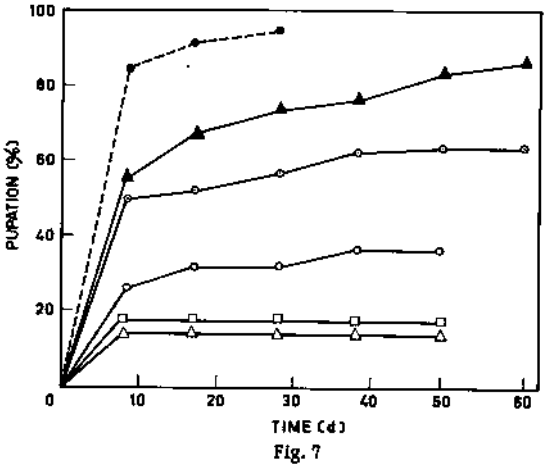

Fig. 7

Effects of post-irradiation temperature of 38°C on pupation

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
—●—●— Control

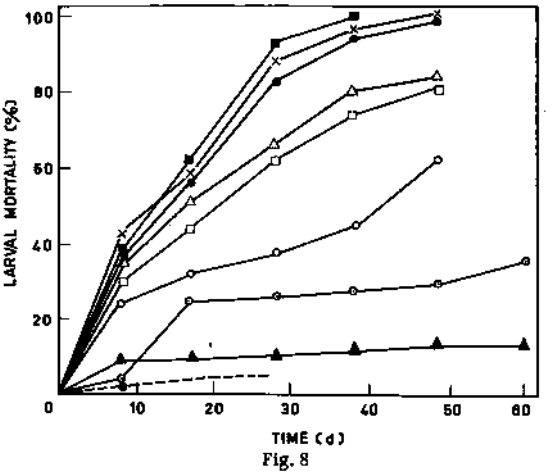

Fig. 8

Effects of post-irradiation temperature of 38°C on larval mortality

▲ 5 krad
⊙ 6 krad
○ 8 krad
□ 10 krad
△ 12 krad
● 14 krad
× 16 krad
■ 18 krad
—●—●— Control

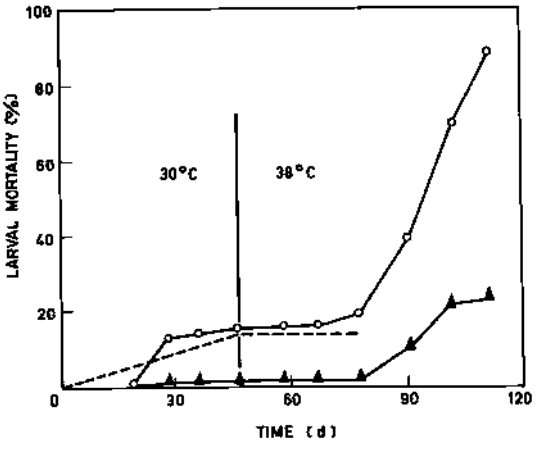

Fig. 9

Effect of higher temperature (38°C) on irradiated larvae maintained for long periods at low temperature

▲ 5 krad
○ 8 krad
--- Control

appear to contribute to this differential susceptibility between male and female. Among these the one that seems most plausible is the body volume. The average body volume of the female is approximately three times that of the male; hence it is presumed that the total energy deposited is correspondingly more in the female than in the male. COLE, M.M. et al. [7] determined the LD50 of insects of different body sizes. According to them the LD50 decreased with the increase in body size. It is therefore reasonable to expect a greater biological damage for the same dose in the female than in the male. Another factor which may contribute to the damage differential is probably the oxygen tension of the tissues. Preliminary studies on oxygen uptake by males and females have shown that the female consumes more oxygen than the male. If the oxygen tension of the tissue is also greater in the female than in the male, it is possible that the biological damage in the female is also more severe, since higher oxygen tension is known to enhance radiation damage [1, 10]. Only a detailed investigation would throw more light on these and other factors which are also involved in enhancing radiation damage in the females. Such studies are in progress.

Irradiation of the larvae at different instars has shown that there is a progressive increase in resistance to radiation as larval development proceeds. The first-instar larvae could not develop any further when the dose applied was 6 krad; 50% mortality occurred on the 20th day after irradiation. On the other hand, when the larvae above third instar were exposed to a dose of 8 krad, 50% mortality was observed on the 43rd day. Pupation was completely inhibited at 6 krad in the first instar, while the corresponding dose for complete pupal inhibition was 14 krad.

BALDWIN, W.E. [2] has shown that the lethal effects of X-rays can be drastically modified by post-irradiation temperature. This he observed in Dahlbominus fascipennis, which was first exposed to X-rays and im-

mediately thereafter transferred to higher temperature for a short time. He concluded that irradiation in some way or the other sensitized the insect to heat which was normally sublethal. When this process was reversed, i.e., X-ray treatment following heat, a negligible killing occurred. In contrast to this, PENDLEBURRY, J.B. and BANHAM, E.J. [12] found in Sitophilus granarius that heating before irradiation sensitized the insect at doses of 5100 and 7700 rad, though at doses above 7700 rad complete kill was obtained within fourteen days when the post-irradiation temperature was 30°C. From our studies it is apparent that post-irradiation temperature does modify radiation response, as is evidenced from the different LD50 obtained for each temperature treatment (Table IV). The arrest of growth noticed at 30°C is associated with a fall in metabolic rate. BURGES, H.D. [4] has shown that the level of respiration of the diapause larvae of T.granarium is considerably lower than that of the normal ones. Since it is known that high oxygen tension can enhance radiation damage, it is likely that the increase in LD50 observed in the "diapausing" larvae may be due to, among other factors, the low oxygen tension in its tissues.

It is evident from the data obtained that, within certain limits, the stage at which the radiation damage manifests itself is largely dependent on the ambient temperature. In the irradiated larvae maintained at 30°C pupation was arrested above a dose of 8 krad. In those maintained at 34°C and 38°C, doses above 10 and 12 krad respectively were required to bring about the same effect. It is well known that reduction in ambient temperature prolongs the larval stage. This is reflected in this study by the fact that at low temperature mortality occurred in the larval stage itself, whereas at higher temperature, as a result of accelerated development, it was seen in the pupal stage. Though pupation occurred at these dose levels, adult emergence was completely inhibited at all doses above 6 krad irrespective of the temperature treatment. In all cases where adult emergence occurred, the adults were found to be sterile.

Though most of the studies carried out by earlier workers have been on the influence of temperature on the radiation susceptibility, no attempt has previously been made to study the effects of radiation on "diapausing" insects. It has been shown that when hibernating mammals such as marmots [14] and squirrels [9] were irradiated in the hibernating stage, mortality was greatly reduced; but when the survivals were awakened the animals died after the same period of sickness as non-hibernating controls. In our studies too, when the larvae were irradiated and maintained in the "diapausing" state, the larval mortality was remarkably low; but when the "diapause" was broken by increasing the temperature, radiation damage began to manifest itself (Fig. 5). This shows that the development of radiation damage was postponed but by no means prevented by "diapause".

SUMMARY

Different stages in the development of Khapra beetle were exposed to various doses of gamma radiation and their effects recorded. It was observed that the females were more susceptible to gamma radiation than the males.

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DISCUSSION

P. NARDON: You state that an increase in the lethal effect of irradiation was observed when the temperature was raised. Might this not be due to the fact that irradiation interferes with the metabolism of the insect? Since insects do not regulate their own temperature, if you raise the external temperature you increase their rate of metabolism. It is not surprising under such conditions that the primary effects of irradiation should also be accelerated. Could you comment on this, please?

K.K. NAIR: You are quite right. It is true that at low temperatures the metabolic rate is also low. When an insect is irradiated in this state, the radiation damage does not manifest itself very rapidly. On the other hand, if the external temperature is raised, it accelerates the metabolic rate, which results in the manifestation of radiation damage.

E.E. TURTLE: The theory that the lower susceptibility of T. granarium at lower temperatures is a consequence of the lower metabolic rate at lower temperatures is supported by the fact that the insect is also less susceptible to fumigants at these temperatures. Some very detailed work is being done to find out for one or two fumigants what is the (concentration × time) needed to kill the various stages of this insect. There again, as the temperature is lowered you need a much higher (concentration × time) to get the same kill, and I think this is explainable by assuming that the effect is due to the lower metabolic rate at lower temperatures.

RADIOSENSITIVITY OF VARIOUS STAGES OF CALLOSOBRUCHUS CHINENSIS L.

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Abstract — Résumé — Аннотация — Resumen

RADIOSENSITIVITY OF VARIOUS STAGES OF CALLOSOBRUCHUS CHINENSIS L. The effects of irradiation on the eggs, larvae, pupae and adults of Callosobruchus chinensis L., a destructive pest of leguminous seeds, have been studied. Since the entire life-cycle except the egg and adult stages of this insect is passed in the seed itself, control by conventional means is very difficult.

Insects were obtained from local grain shops and reared in an incubator in the laboratory on moong (Phaseolus mungo L.) seeds at a temperature of 29 ± 1°C and a humidity of 70-75%. Under these conditions the insect completed its life-cycle in 18-22 d.

A 1-c iridium-192 source was used for initial experiments in irradiation. Later this was increased to 4 c. Exposures were made at very close range and the dose-rates were calculated on the basis of a measurement made at 50-cm distance with a Victoreen condenser r-meter.

Eggs were irradiated at a distance of 0.5 cm from the source and larvae, pupae and adults at 1.0 cm from the source, the respective dose-rates being 80 kr/h at 0.5 cm and 20 kr/h at 1 cm for a 4-c source.

It was found that a dose of 15 krad gave 100% mortality in the case of eggs. Different doses below this level gave somewhat variable results and it is possible that there is a sensitive stage for a short period during the first 24 hours of development of the eggs.

In the case of 8-day-old larvae 100% mortality was obtained with a dose of 20 000 rad. The pupae seem to be less radiosensitive than the eggs or larvae and doses of 47 000 rad were needed to give 100% mortality.

Doses of 42 000 rad "sterilized" the males and females in the sense that, though mating took place after irradiation, the eggs produced when either of the parents had been irradiated with a dose of 42 000 rad did not hatch. Production of "sterile" eggs continued even when either or both parents had been exposed to doses of 67 000 rad - the highest dose tried.

RADIOSENSIBILITÉ DU CALLOSOBRUCHUS CHINENSIS L. AUX DIVERS STADES DE SON DÉVELOPPEMENT. On a étudié les effets des rayonnements sur les œufs, les larves, les pupes et les adultes du Callosobruchus chinensis L., insecte nuisible aux graines de légumineuses emmagasinées. Il est très difficile de lutter contre cet insecte par les moyens classiques, du fait qu'il passe toute la période larvaire et la pupation dans la graine elle-même.

Des insectes obtenus dans des graineteries locales ont été élevés en laboratoire, dans un incubateur, sur des graines de mungo (Phaseolus mungo L.), à une température de 29 ± 1°C et un degré d'humidité de 70 à 75%. Dans ces conditions, le cycle vital de l'insecte est de 18 à 22 j.

Pour les premières expériences d'irradiation, on a utilisé une source d'iridium-192 de 1 c. Son intensité a ensuite été portée à 4 c. L'exposition a été effectuée à une très faible distance; le débit de dose a été calculé d'après une mesure faite à 50 cm avec un dosimètre à condensateur de Victoreen.

Les œufs à irradier ont été placés à 0,5 cm de la source et les larves, pupes et adultes à 1 cm, l'intensité de dose étant de 80 kr/h et de 20 kr/h, respectivement, avec la source de 4 c.

Une dose de 15 000 krad a provoqué une mortalité de 100% dans le cas des œufs. Diverses doses de plus faible intensité ont donné des résultats quelque peu variables; il est possible qu'il existe un stade sensible pendant une brève période des premières 24 h du développement des œufs.

Une dose de 20 krad a provoqué une mortalité de 100% des larves âgées de 8 j. Il semble que les pupes soient moins radiosensibles que les œufs ou les larves; il a fallu des doses de 47 krad pour obtenir une mortalité de 100%.

Des doses de 42 krad ont provoqué la stérilisation des mâles et des femelles, en ce sens que les œufs produits après l'irradiation de l'un des parents ne sont pas parvenus à éclosion. La ponte d'œufs «stériles» a continué même après que l'un des parents ou les deux aient été exposés à des doses de 67 krad, maximum appliqué dans ces expériences.

ЧУВСТВИТЕЛЬНОСТЬ К ОБЛУЧЕНИЮ У РАЗЛИЧНЫХ СТАДИЙ CALLOSOBRUCHUS CHINENSIS L. Было изучено воздействие облучения на яйца, личинки, куколки и взрослые особи Callosobruchus chinensis L. - вредного насекомого, поражающего запасы бобовых культур. Так как весь жизненный цикл этого насекомого, за исключением времени пребывания в состоянии яйца и взрослой особи, протекает внутри самого боба, наблюдать за этими насекомыми обычными средствами чрезвычайно трудно.

Насекомые были взяты из местных зерновых складов и выращивались в лабораторном инкубаторе на семенах мунго (Phaseolus mungo L.) при температуре в 29°C ± 1°C и при влажности в 70 - 75%. В этих условиях насекомые завершали свой жизненный цикл за 18 - 22 суток.

Для первоначальных экспериментов с облучением был использован источник с иридием-192 мощностью 1 кюри. В дальнейшем мощность была увеличена до 4 кюри. Облучение производилось на очень близком расстоянии, и дозы подсчитывались на основании измерений, сделанных на расстоянии 50 см при помощи конденсаторного дозиметра типа Викторин.

Яйца облучались на расстоянии 0,5 см от источника, а личинки, куколки и взрослые особи - на расстоянии 1,0 см от источника, причем соответствующие дозы были 80 000 рад/час на расстоянии 0,5 см и 20 000 рад/час на расстоянии 1 см для источника мощностью 4 кюри.

Было обнаружено, что для яиц доза 15 000 рад давала 100-процентную смертность. Разные дозы ниже этого уровня давали довольно различные результаты, и представляется возможным, что во время первых 24 часов развития яиц имеется непродолжительная чувствительная стадия.

Для личинок в возрасте 8 суток 100-процентная смертность наступала при дозе 20 000 рад. По-видимому, куколки менее чувствительны к облучению, чем яйца или личинки, и для достижения 100-процентной смертности были необходимы дозы в 47 000 рад.

Дозы в 42 000 рад "стерилизовали" как самцов, так и самок в том смысле, что хотя после облучения и происходило спаривание, но из отложенных яиц потомство не развивалось, когда один из родителей подвергался облучению дозой в 42 000 радов. Откладывание "стерильных" яиц продолжалось даже в тех случаях, когда один из родителей или они оба подвергались облучению дозой в 67 000 рад, которая была наивысшей опробованной дозой.

RADIOSENSIBILIDAD DE LAS DIVERSAS FASES DE LA METAMORFOSIS DEL CALLOSOBRUCHUS CHINENSIS L. Los autores han estudiado los efectos de la irradiación sobre huevos, larvas, ninfas e imagos del Callosobruchus chinensis L., insecto que ataca a las semillas de leguminosa almacenadas. Como todo el ciclo de desarrollo del insecto - exceptuando el huevecillo y las fases adultas - tiene lugar en el interior de la semilla, es muy difícil combatirlo por medios convencionales.

Los insectos se recogieron en almacenes de granos y se cultivaron en una incubadora del laboratorio, con semillas de Phaseolus mungo L., a una temperatura de 29°C ± 1° y una humedad de 70 a 75%. El ciclo de desarrollo del insecto duró de 18 a 22 d.

Para los experimentos iniciales de irradiación, se empleó una fuente de iridio-192 de 1 c cuya intensidad se aumentó más adelante a 4 c. Las irradiaciones se efectuaron a muy corta distancia y la intensidad de las dosis se calculó a base de una medición efectuada a 50 cm de distancia con un contador Victoreen, de condensador, calibrado en roentgens.

Los huevecillos se irradiaron a medio centímetro de la fuente, y las larvas, ninfas e imagos a un centímetro; la intensidad de la dosis con la fuente de 4 c fue de 80 kr/h en el primer caso y de 20 kr/h en el segundo.

Se pudo observar que con una dosis de 15 000 rad se obtenía una mortalidad del 100% entre los huevecillos. Con dosis inferiores se obtuvieron resultados variables, y es posible que durante un corto período de las primeras 24 h de desarrollo de los huevecillos haya una fase de mayor sensibilidad.

Con larvas de ocho días, la mortalidad total se obtuvo tras una dosis de 20 000 rad. Las ninfas parecen ser menos sensibles que los huevos y las larvas, y se precisaron dosis de 47 000 rad para alcanzar el mismo resultado.

Una dosis de 42 000 rad «esterilizaba» a los machos y a las hembras en el sentido de que, aunque copularan después de la irradiación, los huevos producidos cuando uno cualquiera de los padres había sido irradiado con esta dosis resultaban hueros. La producción de huevos «estériles» continuó incluso cuando se expuso a uno o a ambos padres a dosis de 67 000 rad, que fue la más intensa que emplearon los autores.

INTRODUCTION

Beetles belonging to the family Bruchidae (order Coleoptera) are very destructive to stored lentils in Turkey, Iran, Pakistan and the Middle and

Far East [1,2,3]. The genera Bruchus and Callosobruchus are particularly serious, and moderate to heavy infestations have been reported in the region. The insects lay their eggs on the outside of the seed and the larvae bore directly into the grains, where they feed on the inside, pupate and emerge as adults by making a hole in the seed coat. Since the life-cycle is a short one – under ideal conditions about 18 - 22 d for Callosobruchus chinensis – there are several generations in a year and the damage is consequently heavy.

Because the insects pass a major portion of their life-cycles inside the seed, conventional means of control are not very successful.

Irradiation offers a promise of control and in view of the paucity of literature on the Bruchidae family, we thought it useful to work out the radiosensitivity of different stages of Callosobruchus chinensis.

IRRADIATION SOURCE AND DOSE RATE

A source of Ir^{192} was used for irradiation. To start with we had a source of about 1 c, but later this was replaced by one of 4.4 c. The source used is by no means ideal for this type of work because, firstly, it gives a whole spectrum of γ-rays and some β-rays [4]; secondly, it has a short half-life; and thirdly, because of its low strength, it is necessary to give exposures at a very close range and consequently the dose-rate cannot be accurately determined.

The dose-rate was obtained in two ways: firstly it was calculated from the 4π flux at 5 cm from the source, assuming a 2×2-mm source to be a point source; secondly it was measured by a Victoreen r-meter at a distance of 50 cm from the source and then calculated at 5 cm distance by the inverse-square law. The two figures were within 10% of each other.

The calculated dose-rate for a 4.4-c source is 820 rad/h at 5 cm. By applying the inverse-square law, which will hold true approximately, the dose at 1 cm is about 20 000 r/h and at 0.5 cm 80 000 r/h.

The measured dose rate at 50 cm distance for the same source is 8.8 r/h and therefore at 1 cm 22 000 r/h.

The dose-rate due to β-radiation has been neglected in view of the self-absorption in the source, β-absorption in the specimen container and β-absorption by the seeds themselves.

MATERIALS AND METHOD

Callosobruchus chinensis L. [5] adults were obtained from local grain shops in the town and were reared in an incubator in the laboratory on moong (Phaseolus mungo Linn.) seeds at a constant temperature of 29 ± 1°C and a humidity of 75 - 80%. While mentioning the incubator temperature, it is necessary to point out that the temperature of the infested grain is not always the same as the temperature of the incubator. Seed temperatures up to 35°C have been recorded while the incubator remained at a constant temperature of 29 ± 1°C.

To obtain eggs, clean Phaseolus mungo seeds were placed with adult Callosobruchus at 4 p.m. in the incubator and the eggs laid overnight were

irradiated at 9 a. m. the next morning. The eggs thus irradiated were up to 17 h old.

Eight-days-old larvae, the age being counted from the day the egg was laid, were irradiated in the intact seed, and so were the pupae.

RESULTS AND DISCUSSION

Table I gives the results of irradiation of eggs at different doses within 30 d. Irradiation at doses of 2 000 r gave variable results and mortalities varying from 18 to 26% were obtained. After making corrections for the

TABLE I

RADIOSENSITIVITY OF EGGS OF CALLOSOBRUCHUS CHINENSIS L.

Radiation dose (rad)	Mortality (%)
0 (control)	3.8
2000	18 - 26
5000	38
10000	58
15000	100

control, we still find this variation statistically significant and feel that this is due to the variation in age of the eggs. Possibly the radiosensitivity differs at different levels of development of the egg.

Table II shows the effect of γ - irradiation on 8 - d - old larvae of Callosobruchus. In the case of larvae, 100% mortality has been obtained by a dose of 20 000 rad.

Pupae seem to be less sensitive to γ - irradiation than either eggs or larvae, and Table III shows the results of irradiation of the pupae. To obtain 100% mortality in this case, a dose of 47 000 rad is required.

Unmated males and females emerging overnight were irradiated separately and after irradiation were paired as follows:

1. Irradiated female and normal male.
2. Irradiated male and normal female.
3. Irradiated male and irradiated female.
4. Normal male and normal female (control).

Doses of 42 000 rad were needed to "sterilize" both the males and females. When either sex exposed to this dose was paired with a normal adult of the opposite sex, eggs were laid on the seeds but the eggs failed to hatch.

Exposures at higher doses were tried and the highest dose-rate given was 67 000 r. Even at this dose the females did not stop oviposition, though the eggs, as with the lower doses mentioned above, were all sterile.

TABLE II

MORTALITY OF LARVAE OF CALLOSOBRUCHUS CHINENSIS L. AT DIFFERENT DOSES OF γ - IRRADIATION

Radiation dose (rad)	Mortality (%)
0 (control)	3.8
10 000	70
15 000	98
20 000	100

TABLE III

MORTALITY OF PUPAE OF CALLOSOBRUCHUS CHINENSIS L. AT DIFFERENT DOSES OF γ - IRRADIATION

Radiation dose (rad)	Mortality (%)
0 (control)	0.8
8 000	50
12 000	54
47 000	100

This is only a preliminary study and the present results have been presented to show that simple irradiation studies in the case of stored-grain pests can be carried out with small sources.

We are now in the process of installing a 100-c Co^{60} source which will enable us to continue this work on a larger scale.

ACKNOWLEDGEMENTS

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DISCUSSION

D. A. CROSSLEY: Did you observe any difference in duration of the larval or pupal stages following irradiation?

M. S. QURAISHI: No, this could not be done because either the insects emerged as adults during the period of observation or the seed was broken open to expose the insect.

K. K. NAIR: Could you tell me the technique of your irradiation experiments?

M. S. QURAISHI: The insects and seeds were put into open tubes with cellophane on one side and pressed by means of a small cord against the cellophane.

K. K. NAIR: Do you feel that by immobilizing the insects on cellophane tape the radiation response will be different from what you would get if they were irradiated in an active state?

M. S. QURAISHI: The small source we were using was not a very good source to use for this purpose, I agree, and we shall continue this work with the bigger source that we have now.

DISINFESTATION OF DRIED FIGS BY GAMMA RADIATION

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Abstract — Résumé — Аннотация — Resumen

DISINFESTATION OF DRIED FIGS BY GAMMA RADIATION. Research has been carried on for many years on the disinfestation of stored agricultural products. Recently gamma radiation has been successfully applied in such investigations. Dried figs are among the main export products of Greece. Serious damage is caused by insect attack each year.

The research work aimed at: (1) Determining the doses of radiation required to destroy certain species of insects attacking dried figs at their various stages of development, or to destroy their eggs and thus prevent reproduction; and (2) Determining the physical and chemical changes of the texture of dried figs caused by radiation.

Studies have been made on the following species: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis and Lasioderma serricorne.

The optimum doses required are discussed. At these doses, irradiation damage does not become apparent in the texture of the figs.

DÉSINSECTATION DES FIGUES SÈCHES PAR LES RAYONS GAMMA. Des recherches sur la désinsectation des produits agricoles en magasin sont en cours depuis de nombreuses années. On a récemment utilisé avec succès les rayons gamma dans ces études. Les insectes font chaque année de grands ravages dans les figues sèches, qui sont l'un des principaux produits d'exportation de la Grèce.

L'objet des recherches était le suivant: 1. Déterminer les doses de rayonnements nécessaires pour détruire certaines espèces d'insectes qui, à différents stades de leur développement, attaquent les figues, ou détruire leurs œufs et les empêcher ainsi de se reproduire; 2. Déterminer les modifications physiques et chimiques que les rayonnements produisent dans la texture de la figue sèche.

Les travaux ont porté sur les espèces suivantes: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis et Lasioderma serricorne.

Le mémoire étudie les dises optimum d'irradiation, qui ne provoquent aucun dommage visible dans la texture de la figue.

ДЕЗИНФЕСТАЦИЯ СУШЕНОГО ИНЖИРА С ПОМОЩЬЮ ГАММА-ИЗЛУЧЕНИЯ. В течение многих лет проводятся исследования по вопросам дезинфестации сельскохозяйственных продуктов в хранилищах. Недавно гамма-излучение было успешно применено при проведении таких исследований. Сушеный инжир составляет один из основных продуктов экспорта Греции. Ежегодно насекомые наносят серьезный ущерб этому продукту.

Цель исследования: 1) определение доз излучения, необходимых для уничтожения некоторых видов насекомых, поражающих сушеный инжир, на различных стадиях их развития, и для уничтожения их яиц и предотвращения таким образом их размножения; и 2) определение физико-химических изменений текстуры сушеного инжира под действием излучения.

Исследования проводились со следующими видами: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis и Lasioderma serricorne.

Обсуждаются необходимые оптимальные дозы. При этих дозах не наблюдается видимого нарушения текстуры инжира под действием облучения.

DESINFESTACIÓN DE HIGOS SECOS MEDIANTE RAYOS GAMMA. Des hace muchos años se están efectuando investigaciones sobre la desinfestación de los productos agrícolas almacenados. En esas investigaciones se han utilizado recientemente con éxito los rayos gamma. Los higos secos constituyen uno de los principales productos de exportación de Grecia y los insectos ocasionan pérdidas importantes.

Las investigaciones tenían las siguientes finalidades: a) determinar las dosis de radiación necesarias para destruir ciertas especies de insectos que atacan los higos en diversas fases de su desarrollo, o destruir sus huevos impidiendo así que se reproduzcan; b) determinar las modificaciones físicas y químicas que las radiaciones producen en la contextura de los higos secos.

Se han estudiado las especies siguientes: Plodia interpunctella, Ephestia cautella, Carpophilus hemipterous, Oryzaephilus surinamensis y Lasioderma serricorne.

En la memoria se estudian las dosis óptimas de radiación que no producen daños aparentes en la contextura de los higos.

1. INTRODUCTION

Since 1930, the disinfestation of dried figs has been compulsory in Greece. Fumigation is the main method used for the destruction of insects infesting the figs. Until 1952, CS_2 was applied at a dose of 80 g/m^3 of space for a duration of 8 h; later, however, this chemical was replaced by methyl bromide given in special fumigation chambers at a dose of 25 g/m^3 of space for 12 h.

The main disadvantages of fumigation with methyl bromide are:

(a) Methyl bromide is an odourless and very toxic gas, so that special precautions need to be taken during its use and the figs must be well aerated after treatment;

(b) To ensure good penetration by the fumigant, the figs must be treated before packing;

(c) Fumigation kills adults and larvae but has a limited effect on the viability of the eggs, so that it is necessary to repeat the treatment after a certain time of storage, in order to kill the larvae which have emerged in the meantime from the unaffected eggs.

In the search for a more effective method of disinfesting dried figs, we decided to use the radiation method, which had proved satisfactory in the treatment of stored agricultural products. To satisfy our needs, the method of treating figs with gamma radiation should meet the following requirements:

(a) It should be possible to treat the figs after packing;

(b) The method must be more efficient than the previous ones in that not only adults and larvae but also all the eggs must be killed;

(c) There must be no side-effects on the texture, appearance and nutritional value of the treated products; and

(d) The large-scale application of the method must be physically and economically feasible.

2. MATERIALS AND METHODS

In view of the very large number of published papers dealing with various aspects of insect disinfestation of stored agricultural products by means of ionizing radiation no attempt will be made here to review the pertinent literature (a short Bibliography is appended). In this preliminary paper we describe briefly the methods and the results so far obtained in our own experiments.

The research work was divided into two parts. In the first part of our experiments, we examined the effects of gamma radiation on the insects infesting Greek figs. To obtain large numbers of these insects, very heavily infested figs were collected from Calamata, the main export centre for this

product, and placed in suitable insectaria. They served also as experimental media for rearing the insects.

The species found in the examined figs were identified as:

(a) Plodia interpunctella (Lepidoptera)
(b) Ephestia cautella (Lepidoptera)
(c) Carpophilus hemipterous (Coleoptera)
(d) Oryzaephilus surinamensis (Coleoptera)

All radiation exposures were made in the gamma irradiation facility of the Democritus Nuclear Research Centre, where a 500-c Co^{60} source was avail-
All radiation exposures were made in the gamma irradiation facility of the Democritus Nuclear Research Centre, where a 500-c Co^{60} source was available. The dose-rate of the source was 30-40 krad/h. Calibrations were made by the Fricke chemical dosimetry method, using ferrous sulphate solution.

Eggs, larvae and adults were irradiated separately in groups of 50 for the eggs and of 20 for the other metamorphic forms at various doses ranging from 25 to 400 krad. Controls were placed under the same conditions except for irradiation. Counts of dead and living insects were made every day, together with studies on developmental and reproductional changes.

All insect cultures were maintained in the incubator under a constant temperature of 28°C and at a relative humidity of 40%.

3. EXPERIMENTAL RESULTS

(a) Eggs

In general, doses below 25 krad do not seriously affect the hatching of the eggs of the different species. The dose of 25 krad reduces hatching of

TABLE I

MORTALITY AND RELATED RESPONSES TO GAMMA RADIATION OF INSECTS INFESTING FIGS

SPECIES	EGGS		LARVAE		PUPAE	ADULTS	
	Dose (krad)	Hatch (%)	Dose (krad)	T_{50} (d)	No-eclosion dose (krad)	Dose (krad)	T_{50} (d)
Plodia interpunctella	25	15	100	2	100	100	4
	50	7	250	1		400	1
Ephestia cautella	25	10	100	3	100	150	4.5
	50	2	300	1		400	1
Carpophilus hemipterus	25	12	100	3	100	150	3
	50	5	300	1		400	1
Oryzaephilus surinamensis	25	30	100	2	100	150	3.5
	50	10	250	1		400	1.5

the eggs to 10% for Ephestia, 12% for Carpophilus, 15% for Plodia and 30% for Oryzaephilus. At a dose of 50 krad, hatching is reduced to 2% for Ephestia, 5% for Carpophilus, 7% for Plodia and 10% for Oryzaephilus, but the enclosed larvae in general were not viable. At a dose of 100 krad, hatching was practically nil (Table I).

(b) Larvae

The effect of the radiation on the larval stage is expressed here as "T_{50}", which is the time required for 50% of the irradiated population to die after exposure to a given dose of radiation. For a dose of 50 krad, T_{50} varied between 5 and 6 d, depending upon the age of the larvae and the species. At a dose of 100 krad the value of T_{50} was 2 - 3 d, and a dose of 250 krad reduced this value to 1 d (Table I).

(c) Pupae

In general, there was no eclosion of the pupae after doses of 50 krad and above (Table I).

(d) Adults

At a dose of 50 krad, the T_{50} was between 6 and 8 d, depending upon the species, Oryzaephilus being the most resistant. At a dose of 150 krad the T_{50} was between 3 and 4 d. A dose of 400 krad killed all insects in less than 48 h (Table I).

4. EFFECTS OF IRRADIATION ON THE PRODUCTS

In the second part of our experiments, we examined the effects of various doses of gamma radiation upon the colour and the sugar content of the dried figs, as a preliminary check on possible side-effects of irradiation on the texture and appearance of the treated figs.

Colour

For the colour estimation, we used the following standard method. 5 g of figs were extracted with a mixture of equal volumes of alcohol and 10% trichloroacetic acid at a constant temperature of 45°C. The extract was mixed with 0.5 g Supercell and filtered. The colour of the filtrate was then determined in a Lumetron colorimeter in 420 nm, using as blank a mixture of alcohol and trichloroacetic acid. The results are expressed as per-cent transmission in Table II.

As shown in the Table, no significant changes of colour result after irradiation with doses ranging between 25 and 400 krad.

Sugars

For sugar estimation we applied the Lane and Eynon method, which permits determination of total and reducing sugars. Table III gives a picture

TABLE II

PHOTOMETRIC ESTIMATION OF THE COLOUR OF DRIED FIGS

(% transmission)

Dose (krad)	Irradiated	Not irradiated
25	33	40
50	33	40
100	33	40
150	34	41
200	35	39
250	32	38
300	36	43
350	35	40
400	38	43
500	33	40
600	22	35

TABLE III

SUGAR ESTIMATION OF IRRADIATED FIGS

Dose (krad)	Total (%)	Reducing (%)	Non-reducing (%)
Blank (average)	52	46.2	5.8
25	52	46.5	5.5
50	49.25	45.44	3.81
100	49.25	45.44	3.81
150	49.25	45.44	3.81
200	49.10	45.40	3.70
250	49.10	45.40	3.70
300	48.90	45.20	3.70
350	49.00	46.28	3.72
400	48.80	45.40	3.40

of the doses employed and the changes produced in sugar content after irradiation of the figs.

Table III shows that the total sugar content is not significantly affected by radiation; the slight difference between the total sugar contents of the

blank and of the irradiated figs is probably due to some decomposition of carbohydrates caused by radiation. There is also a slight diminution of non-reducing sugars, which is due to hydrolytic reactions caused by radiation.

5. CONCLUSIONS

Preliminary experiments have shown that the disinfestation of figs by gamma radiation seems to be a very promising treatment, since at the moderate doses of 100 - 200 krad needed for the destruction of the different stages of the infesting insects, no significant changes result in the texture, appearance and nutritive value (related to carbohydrates) of the figs.

Of course many problems are still to be solved and much work has to be done to overcome the difficulties associated with the large-scale application of this method. Some of these problems are the economical feasibility of the method for commercial use, the development of new packaging materials, and better conditions of storage in order to prevent re-infestation of irradiated figs.

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DISCUSSION

P. PELEGRIN: Is there any legislation in Greece to control the irradiation of foodstuffs?

A. PAPADOPOULOS (on behalf of the author): No, there is no such legislation. I think we shall have to carry out tests for several years to be sure the method is safe. I have heard something about the use of irradiated potatoes in the USSR and to a limited extent in Canada.

P. PELEGRIN: Yes. Potatoes may be irradiated in Canada and the USSR, I believe, and in the United States the irradiation of bacon with 4500 Mrad has been authorized since 15 February 1963. That is quite a high dose which probably represents a new direction in the conservation of food products by irradiation.

M. FRIED: I might add that disinfestation of grain by irradiation is now under consideration in the United States and a decision is expected shortly.

B. DARIS: Have you experimented with dried figs of varying water content?

A. PAPADOPOULOS: No.

B. DARIS: Did you have any trouble through the appearance of sugar crystals at high humidities? I have made certain experiments on raisins and I found that with high water contents of about 20-22% sugar crystals soon appear and this modifies the behaviour of the dried fruit.

A. PAPADOPOULOS: We had no such trouble. The fact that you tested without a plastic package might explain your difficulties.

SYMPOSIUM ON THE USE AND APPLICATION OF RADIOISOTOPES AND RADIATION IN THE CONTROL OF PLANT AND ANIMAL INSECT PESTS

Athens, 22-26 April 1963

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AUTHOR INDEX

Numerals underlined refer to the first page of a paper by the author concerned.
Other numerals denote the page-numbers of other references, principally discussion comments.
Literature references have not been indexed.

Andreev, S.V.: 115, 127, 128 129, 130, 131, 132
Arroyo, Varela, M.: 83
van Asperen, K.: 144, 153, 154, 265, 311
Baccetti, B.: 356, 387, 412, 424
Baines, B.D.: 385, 429
Bantzios, D.N.: 105
Binggeli, M.-H.: 411
Boroughs, H.J.: 130, 355
von Borstel, R.C.: 53, 253, 287, 310-2, 367, 383-4, 385, 429
Burnett, G.F.: 152, 153, 184, 192, 221
Casida, J.E.: 83, 184, 210, 220, 223, 238, 239, 265
Catrina, I.: 211, 220-221
Cavalloro, R.: 22, 440-1
Constantinescu, El.: 211
Constantinescu, O.: 211
Constantinescu, V.: 211
Courtois, G.: 5, 41
Crossley, D.A., Jr.: 40, 41, 43, 53, 54, 113, 143, 247, 484
Daris, B.: 129, 247, 491
DeLattre, R.: 85, 92, 312
de Dominicis, R.: 387
Feron, M.: 22, 104, 331, 355-6, 362-3
Filatov, G.V.: 249, 253
De Fluiter, H.J.: 129
Fried, M.: 54, 131, 167, 311, 491
Fuzeau-Braesch, S.: 40-41, 42, 265, 287, 289, 297, 440-1
Giles, R.H., Jr.: 55
Gösswald, K.: 25, 241
Hagen, K.S.: 279, 333, 355, 356
Halberstadt, J.: 23, 42, 142-3, 253, 280
Hascoët, M.: 166, 195, 210, 238
Hassett, C.C.: 99, 239, 265
Heath, D.F.: 152, 185, 192, 193, 266
Horber, E.: 248, 297, 313, 331, 385
Huluţă, C.: 211
Huque, H.: 53, 238, 312, 455, 463
Joftes, D.L.: 155, 166, 167
Karandeinos, M.G.: 101
Kartashov, P.A.: 249
Kljajić, R.: 129-130
Kloft, W.: 21, 22, 25, 40, 41-2, 53, 83, 98, 183, 241, 247, 248, 280, 297, 331, 412
Krimbas, C.: 383
Laviolette, P.: 310, 431
Lecomte, J.: 5, 22-23, 40, 103, 104
Lewis, C.T: 104, 135, 143-4, 145, 247
Lindquist, D.A.: 171
Manoto, E.C.: 443
Martens, B.K.: 115
Meltzer, J.: 239
Metin, M.: 479
Miles, J.W.: 153, 183, 310
Molchanova, V.A.: 115
Mourikos, P.A.: 105
Mutin, M.I.: 249
Nadel, D.J.: 114
Nair, K.K.: 127, 166, 310, 384, 425, 429, 465, 477, 484
Nardon, P.: 310, 384, 412, 431, 440, 441, 453, 477
Ogle, J.R.: 144, 193, 297
Orphanidis, P.S.: 101, 104, 424

Papadopoulos, A.: 490, 491
Papadopoulou, C.P.: 453, 485
Pelegrin, P.: 490
Pelekassis, C.E.D.: 105, 113, 114, 356
Peterle, T.J.: 55, 83, 84
Petersen, H.I.: 239
Phillips, F.T.: 143, 147, 152-3, 153-4, 184, 248
de Pietri Tonelli, P.: 131
Plapp, F.W., Jr.: 171
Popa, A.: 211
Quraishi, M.S.: 93, 98, 99, 104, 128, 309, 311, 479, 484
Rahalkar, G.W.: 310, 465
Robbins, W.E.: 184, 269, 279, 280
Saito, T.: 221, 255, 265
Samoilova, Z.A.: 115
Santas, L.: 333
Scarascia, G.-T.: 23, 384, 385, 440
Schmidt, C.H.: 92, 99, 104, 114, 131-2, 193, 220, 310
Schulze, E.-F.: 241
Sekeris, C.E.: 238, 281, 287, 296
Silva, G.M.: 104, 355
Soria, F.: 357, 363
Soultanopoulos, C.D.: 101
Stone, W.E.: 301, 309, 310, 311, 312
Subramanyam, G.: 425
Süss, A.: 310
Thomou, H.: 413, 424
Tonelli, P. de Pietri: See under Pietri
Tsecouras, A.: 333
Turtle, E.E.: 84, 130, 462-3, 477
Uzakov, U.Y.: 249
Viado, G.B.: 332, 443, 453
Zakamyrdin, I.A.: 249

TRANSLITERATION INDEX

Андреев	Andreev
Закамырдин	Zakamyrdin
Карташов	Kartashov
Мартенс	Martens
Молчанова	Molchanova
Мутин	Mutin
Самойлова	Samoilova
Узаков	Uzakov
Филатов	Filatov

SUBJECT INDEX

Abeille
voir "Apis mellifera"
Acanthoscelides obtectus, 122, 131
Acarina, 119
C^{14}-acetate, 280
N-acetyldopamine, 283, 286, 287
Acrididae, 16, 291
Aëdes aegypti, 99, 141, 144, 304, 307
Agrobacterium luteum, 348
Aldrin, 148, 149, 153-4
Alpha particles, 161
Amblyomma americanum, 305
Amiton, 179
Anastrepha ludens, 107, 303, 304, 306 307, 310, 311, 334
Anopheles quadrimaculatus, 304, 306-7, 310
Anopheles stephensi, 95, 96, 98, 99, 309
Ant (see also Formica) 22, 23, 25, 27, 28, 29, 30, 31, 32, 35, 42, 53
Anthonomus grandis, 305, 435
Aphaniptera, 119
Aphid, 22, 37, 177
Aphididae, 119, 129
Apis mellifera, 7, 11, 14, 22, 23, 27, 28, 29, 379
Apis mellifica, 30
Aspergillus flavus, 125
Au
see "Gold"
Autoradiograph, 12, 20

Bayer 8169, 187
Bayer 22408, 176
Bayer 37344, 228
Bayer 39007, 228
Bayer 44646, 228
Beauveria bassiana, 125
Bee
see "Apis mellifera"
Benzene hexachloride, 246
Beta particles (see also "Electrons") 161
Beta sativa, 187
Bidrin, 179, 180, 184
Blattella germanica, 229, 232, 271, 276
Blow-fly (see also "Calliphora erythrocephala" and "Phaenicia sericata") 142, 144
Bombyx mori, 271, 374, 377, 378, 380, 384
Brestan, 188
Bruchidae, 480, 481
Bruchus, 481
Bruchus pisorum, 122, 131
Butanol, 287
Butineuse
voir "Worker"

Cacao theobroma, 186
Cadra cautella (syn. Ephestia cautella) 457, 487, 488
Caesium, 50, 51, 54
Cs^{134}, 48
Cs^{137}, 46, 48, 49, 51, 52, 54, 62, 123, 385
Calandra granaria, 122, 243, 244
Calandra oryzae, 122
Calliphora erythrocephala, 281, 282, 283, 284, 285, 286, 287
Callitroga hominivorax, 315, 414, 458
Callosobruchus, 481
Callosobruchus chinensis, 479-484
Callosobruchus subinnotatus, 458, 460, 462
Camponotinae, 30
Camponotus herculeanus, 31
Camponotus ligniperda, 30, 31
Camponotus spp., 30
Carbamate, 183, 223, 225-236, 239, 253
Carbon
C^{14}, 138, 143, 159, 164, 176, 183, 226-236, 250-1, 271-6, 280, 282-3, 291
Carbon disulphide (CS_2) 486

Carpocapsa pomonella, 378
Carpophilus hemipterus, 487, 488
Caterpillar, 40
Cattle grubs, 176
Centruroides limpidus, 305
Ceratitis capitata, 102,107,303, 308, 334, 357-363, 414
Ceratophyllus fasciatus, 119
Cerium
Ce^{141}, 15
Chemosterilant, 311, 371
Chilo suppressalis, 256-263
Chipman R-6200, 179
Chironomus, 286
Chloridea obsoleta, 124, 132
Chlorine
Cl^{36}, 148, 251
Chlorophos
see "Dipterex, methyl"
C^{14}-cholestanol, 275
C^{14}-cholesterol, 271, 273, 275, 276
Chortophila brassicae, 124-5, 132
Chrysomela knabi, 47, 48, 49
Cobalt
Co^{60}, 8,120,123, 128, 129, 385, 389, 414, 426, 428, 429, 432, 445, 446, 458, 483, 487
Cochliomyia hominivorax, 303, 304, 307, 362, 374
Cockchafer
see "Melolontha vulgaris"
Cockroach, American
see "Periplaneta americana"
Cockroach, Florida
see "Eurycotis floridana"
Cockroach, German
see "Blattella germanica"
Colorado (potato) beetle
see "Leptinotarsa decemlineata"
Coprophagous insects, 176
Co-Ral, 174, 175, 176
Cosmophila, 91
Cotton, 85, 91, 179, 180, 181
Cryotermes brevis, 37
Cryptorrinchus lapathi, 214
Culex tarsalis, 305
Curculionidae, 431, 441
dℓ-cystine-S^{35}, 291, 294, 295, 297
H^3-cytidine, 286
Dacus, 355, 356
Dacus cucurbitae, 303, 306, 308, 311, 334, 414
Dacus dorsalis, 107, 303, 306, 307, 308, 334, 343-4, 414
Dacus oleae, 101, 104, 105, 107-111, 113,114,333-356,387-412, 413-424
Dacus tryoni, 334, 344, 356
Dahlbominus fuscipennis, 475
DDT, 65, 130, 131, 144, 183, 250, 251
DDVP, 253
Demeton, 178, 179, 180, 187, 193, 198, 204, 207, 208
Demeton-S, 195, 197-204, 206, 207, 209
Demeton, thiol isomer, 179
Demeton, thiono isomer, 180
Dermestes maculatus, 271, 275
Dermestidae, 304
Diaspididae, 144
Dieldrin, 138-144, 148, 149, 151, 153-4, 183
Di-iodo-octadecane, 136, 138
Diisopropyl fluorophosphate-P^{32},193
Dimefox, 178, 179, 186, 187
Dimethoate, 175, 178, 179, 180, 181, 253
Dimetilan, 225, 226, 228, 232, 233, 239
Diparopsis watersi, 87, 91, 92
Dipterex, 174, 214-221
Dipterex, methyl (Chlorophos) 252, 253
Disyston, 179, 191, 201
DNA (Deoxyribonucleic acid) 284, 286
Dolichoderinae, 30, 37
Dolichovespula media, 19
Dopamine, 283
Drone, 10, 21, 30
Drosophila, 279, 373, 378, 380, 385, 448
Drosophila melanogaster, 304, 311, 371, 376, 377, 379
Drosophila virilis, 376, 377
Dysdercus, 91, 144

Ecdysone, 283, 284, 286, 287, 296

Electrons (see also "Beta particles") 428, 429
Empusa muscae, 159
Emulsion, nuclear, 160-161, 166-7
Endothion, 195-210
Ephestia
see "Cadra"
Epilachna philippinensis, 446-453
Epilachna varivestis, 238, 304
Epimyrma gösswaldi, 30
C^{14}-ergosterol, 276
Ethyl chloramate, 234
Ethyl methanesulphonate, 384
Eurycotis floridana, 271, 274, 276
Eurygaster integriceps, 96, 97, 119, 128

Famophos, 176
Fe
see "Iron"
Fig, 485-491
Formica cordieri, 28
Formica nigricans
see "F. pratensis"
Formica polyctena, 13, 22, 28-35, 280
Formica pratensis, 28, 37, 38
Formica rufa, 13, 22, 28, 34
Formica spp., 22, 28, 30, 31, 32, 34, 40
Fruit-fly
see "Anastrepha ludens", "Ceratitis capitata", "Dacus cucurbitae", "Dacus dorsalis", "Dacus oleae", "Dacus tryoni", "Drosophila", "Drosophila melanogaster", "Drosophila virilis" and "Trypetidae"

G-23611, 228
Gadfly, 252
Gamma disinfestation, 123
Gamma radiation, 7, 123, 161, 303-5, 308, 315, 363, 413-424, 428, 433-4, 437-8, 440, 443-453, 458-462, 465-477, 481-3, 485-491
Geometridae, 80, 81
Glossina spp., 184
Gold
Au^{198}, 7, 8, 13, 19, 23, 65
Gossypium, 87
Grain weevil
see "Calandria granaria", "Sitophilus granarius"
Grasshopper, 17, 18, 23, 46
Gryllus bimaculatus, 291, 296
GS-13332, 228

H^3,
see "Tritium"
Habrobracon juglandis, 370, 372-375, 377, 379, 384, 388, 429, 436
Hadena sordida, 119, 129
Haematobia irritans, 305
Heliothis, 91, 92
Hercules 5727, 228
Hexadecane, 138, 139, 141, 142, 143, 144
Honey-bee
see "Apis mellifera"
House-fly
see "Musca domestica"

Indium
In^{113m}, 189
Iodine
I^{131}, 28, 32, 33, 37-8, 42, 136, 138, 141, 143
I^{132}, 42
Insecticide, systemic, 173, 176, 211, 220
Iridium
Ir^{192}, 16, 17, 18, 23, 41, 481
Iron
Fe^{59}, 120
Isocyanate-C^{14}, methyl, 226, 227
Isolan, 225, 226, 228, 233

K
see "Potassium"
Kalotermes flavicollis, 36, 37, 38
Khapra beetle
see "Trogoderma granarium"

Lasioderma serricorne, 308, 457
Lasius minutus, 32
Lasius niger, 30
Lasius spp., 30
Lemon, 179, 180
Leptinotarsa decemlineata, 119, 125, 132, 321
Leptocorisa varicornis, 256-263
Leptothorax, 30
Lindane, 316
Locusta migratoria, 22, 291, 292

Malathion, 55, 58, 63-5, 67-8, 76-8, 80, 82-3, 176, 183
Mâle (insectes sociaux)
voir "Drone"
Manganese
Mn^{54}, 65
Marmot, 476
Mediterranean fruit-fly ("Medfly")
see "Ceratitis capitata"
Melanoplus differentialis, 46
Melolontha vulgaris, 313, 315-332, 385
Melon fly
see "Dacus cucurbitae"
Meniscus agnatus, 119
Mephenesin carbamate, 234
Meprobamate, 234
Mercaptophos, 215
Meromyza americana, 297
Metaphos, 121
Metasystox, 214
Methionine-S^{35}, 193, 294, 295, 297
dl-methionine-S^{35}, 291
Methocarbamol, 234
Methyl bromide, 486
Mexican bean beetle
see "Epilachna varivestis"
Mexican fruit-fly
see "Anastrepha ludens"
Microphanurus sp., 97
Millipede, 41
Monerinae, 30
Mormoniella vitripennis, 374
Mosquito
see "Anopheles stephensi"
Musca domestica, 101-3, 141, 144, 227, 232, 238-9, 269, 271-5, 372
Musca domestica vicina, 256-263, 277
Myrmica scabrinodis, 37, 38
Myrmicinae, 30, 37

Naphtol, 227, 230
Narcotics, 119, 287
Necrobia rufipes, 445
Nemeritis canescens, 376
Nephotettix bipunctatus cincticeps, 256-263
Neutrons, 385
Nitidulidae, 81
Noctinidae, 87
Nuclear emulsion
see "Emulsion, nuclear"

Octadecane, 136, 138, 141
Olive fruit fly
see "Dacus oleae"
Oncopeltus fasciatus, 229
Or radioactif
voir "Gold"
Orthotylus virescens, 104
Oryzaephilus surinamensis, 426, 487-8
Oscinis pumilionis, 297
Ostrinia nubilalis, 305, 378

Paranthenae tabaniformis, 214
Parathion, methyl, 180
Paravespula germanica, 19
Paravespula vulgaris, 18-19
Pectinophora gossypiella, 305
Pectinophora malvella, 125, 132
Periplaneta americana, 143, 230, 232, 256-264
Phaenicia sericata, 271
Phaseolus mungo, 481
Phorate, 178-180
Phormia regina, 102-3
Phormia terraenovae, 136
Phosphorus
P^{32}, 10-11, 28, 31-3, 36-7, 40-42, 58, 88-92, 95, 97, 101-2, 104-5, 107-110, 113-4, 120, 122, 128-130, 173-6, 178-180, 183-4, 186-7, 193, 197,

215, 235-6, 252-3, 259, 260, 262-3, 265, 322-4, 358-9
P^{33}, 42
Phytophthora infestans, 188
Pigmentation, 289-297
Pissodes strobi, 371
Planococcus citri, 381
Platyparea poeciloptera, 197, 204, 210
Plodia interpunctella, 457, 487, 488
Polychlorpinene, 251, 252
Polyergus rufescens, 30
Populus robusta, 211, 214, 215, 216, 217, 219, 221
Populus x euramericana
see "P. robusta"
Porthetria dispar, 305
Potassium
K^{40}, 15
Prodenia, 91
Proformica nasuta, 38
Pseudogonia, 129
Pseudomonas savastanoi, 335-6, 347, 348, 356
"Puff", 286, 287
Pyramat, 232
Pyrethrin, 142

Queen, 10, 30, 31, 40

Reine
voir "Queen"
Residue, 62, 83-4, 147-8, 150, 154, 177, 185, 191-2, 198, 200, 202-4, 206, 231
Resistance, 239
Rhagoletis completa, 107, 109
Rhizopertha dominica, 457-460
Rhodnius, 142
Risella 17, 136, 138-142
Risella 33, 138-142
RNA (Ribonucleic acid) 284, 286
Rogor
see "Dimethoate"
Ronnel, 176
Rubidium
Ru^{103}, 15
Ruelene, 175

Salix nigra, 48
Saperda populnea, 214, 219
Sauterelle
voir "Grasshopper"
Schistocerca gregaria, 296
Schradan, 178, 179, 201, 255-265
Sciara coprophila, 374
Scotinophara lurida, 256-7, 259-261, 263
Screw-worm
see "Callitroga hominivorax" and "Cochliomyia hominivorax"
Seed, treatment of, 178, 179
Selenium, 177
Serviformica, 30
Sesamex, 227
Sevin, 225, 227-231, 233, 235, 238, 253
Silkworm
see "Bombyx mori"
Sitophilus granarius, 310, 435, 457, 458, 460, 466, 476
Sitophilus oryzae, 435, 446, 450, 452, 457, 466
Sitophilus sasakii, 310, 431-441
H^3-β-sitosterol, 276, 277
Sn^{113}
see "Tin"
Sodium sulphate - S^{35}, 291, 294, 295
Soldier, 37
Spray (aerial) 64-7, 69, 71-2, 80
Squalene, 20, 21
Squirrel, 476
Sternochetus mangiferae, 308
Stomoxys calcitrans, 305
Strontium
Sr^{90}, 54, 62
Sulphur
S^{35}, 11, 12, 55, 58, 62-4, 82-4, 92, 104, 180, 193, 197, 242, 244-5, 291, 297
Sylepta, 91
Systemic, 121, 173, 176, 179, 181, 211, 220
Systox, 187

Taeniothrips laricivorus, 215
Tantalum
Ta^{182}, 97

TEPA, 311
Termite, 25, 27-8, 35-7
Thiodan, 83, 241-8
Thiophos, 121
Thysanoptera, 119, 129
Thysanura, 280
Tiefenwirkung, 177
Tin
  Sn^{113}, 189, 190, 192
Tribolium, 379, 380, 436
Tribolium castaneum, 138-141, 425-9, 446, 448, 450, 452, 458-460
Tribolium confusum, 376, 377, 426, 450
Triphenyltin, 188-193
Tritium (H^3) 138, 141-5, 159, 164, 226-7, 272, 276-7
Trogoderma granarium, 310, 458, 460, 461, 465-477
Trolene, 253
Trophallaxis, 30, 31, 40
Trophobiosis, 22, 30
Trypetidae, 334, 339, 343, 344, 347, 354, 357
Tryptophane-C^{14}, 291, 293, 294, 295, 296
Tsetse, 184
Tyrosine, 281-3, 291-6

Union Carbide 10854, 228
Urethane, 234
H^3-uridine, 286

Wohlfahrtia magnifica, 118
Worker (social insects) 7, 10, 27, 30, 31, 35, 37, 38, 40

Xenopsylla cheopis, 119
X-radiation, 125, 161, 305, 308, 315, 317-8, 323, 329, 330, 372, 376-8, 385, 433, 436, 440, 457, 475-6

Zectran, 228
$(Zr+Nb)^{95}$, 15, 16

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Corrigenda

E. HORBER: Eradication of white grub (Melolontha vulgaris F.) by the sterile-male technique

p. 316: Opening words of first complete paragraph were omitted; it should begin: "It should be noted that males heavily outnumber females. . . ".

p. 321, sixth line up: for "and the are" read "and they are".

p. 322, last two lines of first paragraph in 214: for "vermiculate" read "vermiculite".

p. 330, 15th line down: for "isotopic dolution" read "isotopic dilution".

BACCIO BACCETTI and RAFFAELLA de DOMINICIS: The effects of gamma radiation on the ovaries of Dacus oleae Gmel.

p. 389, fourth complete paragraph: delete last sentence, beginning "Since research of this kind. . . " and substitute: "This research was performed under IAEA Research Contract No. 169/RB."

pp. 395-406, Figs. 9-20: In each caption the magnification factor should read "6677 diam."

Chairmen of Sessions, Secretariat of the Symposium and list of Participants

p. 493: For "G. PANKRAZIS" read "G. PANTAZIS"
For "G. B. SCHMIDT" read "C. H. SCHMIDT"

p. 498: For "Pankrazis, G." read "Pantazis, G."

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