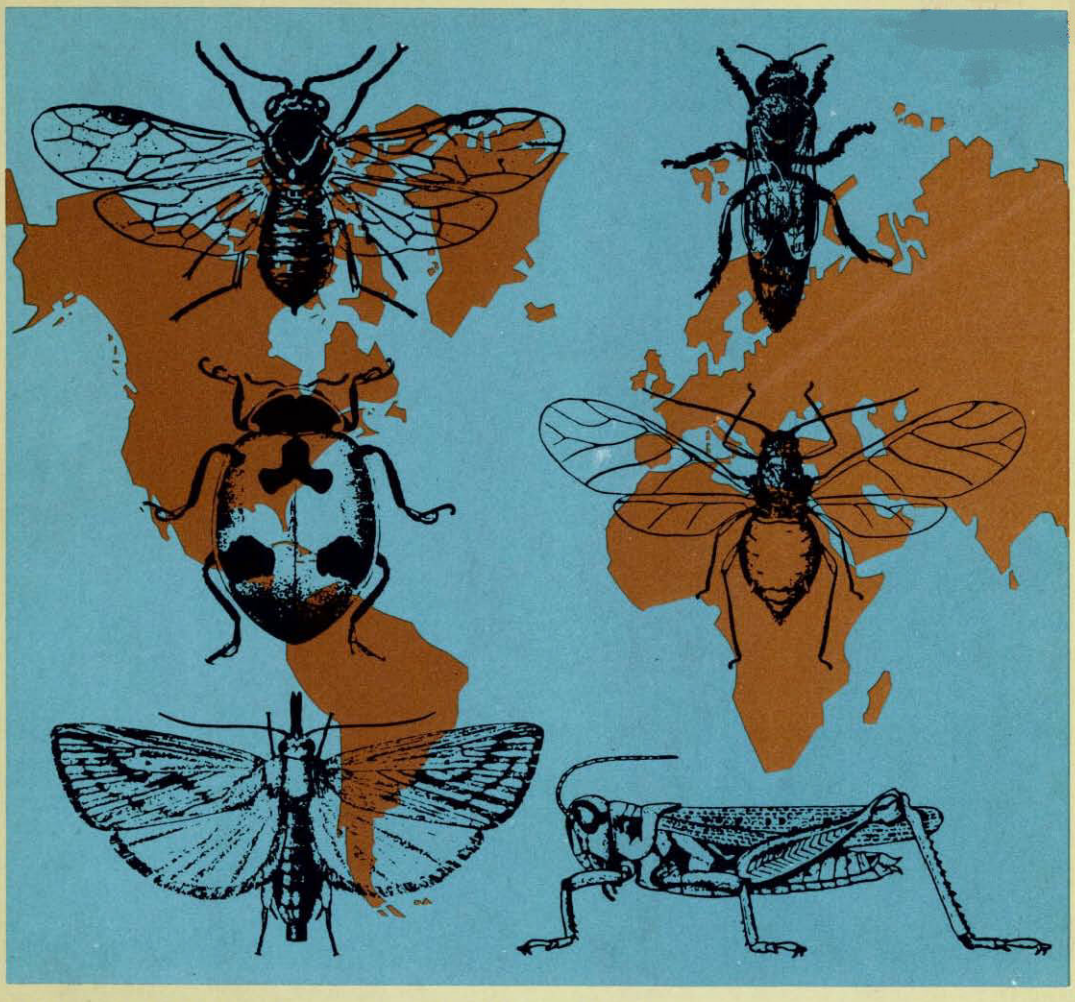

PROCEEDINGS OF A SYMPOSIUM,
VIENNA, 4-8 DECEMBER 1967
JOINTLY ORGANIZED BY THE IAEA AND FAO

ISOTOPES AND RADIATION IN ENTOMOLOGY

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1968

ISOTOPES AND RADIATION IN ENTOMOLOGY

PROCEEDINGS SERIES

ISOTOPES AND RADIATION IN ENTOMOLOGY

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ON THE USE OF ISOTOPES AND RADIATION IN ENTOMOLOGY
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AND THE
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AND HELD IN VIENNA, 4-8 DECEMBER 1967

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ISOTOPES AND RADIATION IN ENTOMOLOGY
(Proceedings Series)

ABSTRACT. Proceedings of a Symposium jointly organized by the IAEA and the Food and Agriculture Organization of the United Nations and held in Vienna, 4-8 December 1967. The meeting was attended by 82 participants from 29 countries and six international organizations.

Contents: Isotope applications - ecology (7 papers); Radiation effect studies - non-genetic (8 papers); Isotope applications - physiology and biochemistry (2 papers); Isotope applications - chemosterilants (2 papers); Sterile-male technique (9 papers); Radiation effect studies - genetic (5 papers); Isotope applications - genetic (1 paper).

Each paper is in its original language (21 English, 11 French, 1 Russian and 1 Spanish) and is preceded by an abstract in English with a second one in the original language if this is not English. Discussions are in English.

(428 pp., 16 × 24 cm, paper-bound, 104 figures)
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ISOTOPES AND RADIATION IN ENTOMOLOGY
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FOREWORD

Insects that are harmful to animals and plants continue to pose one of the most serious problems in agriculture. Despite sophisticated equipment and the continuous development of new techniques, only a few species of harmful insects can be said to be 'controlled'. Agricultural losses from the activity of insect pests amount to millions of dollars annually, and the spreading of plant disease by insect vectors is one of the major causes of famine.

Insecticides have met with only limited success in pest eradication programmes. Many insects develop resistance so that new insecticides or stronger concentrations of those already in existence must be used. Some insecticides adversely affect other components in food chains, thus upsetting ecosystems. The possible accumulation of toxic insecticide residues, which might reach levels harmful to the biota, represents another potential undesirable effect. Nevertheless, such pesticides play an important role in the control of arthropod pests. Isotopically tagged pesticides permit the scientist to follow their distribution and concentration during metabolism in the organism or in higher levels of ecological classification.

The nuclear era has provided man with new and versatile tools for controlling insect pests and for studying various aspects of entomology that were hitherto unheard of or considered impractical or impossible. Radiation and isotopes contribute significantly to our knowledge and understanding of various disciplines related to entomology, such as genetics, physiology, behaviour and ecology. This knowledge is essential for the scientist to plan the most effective and economical ways, not only to eliminate insect pests, but also to propagate beneficial insects.

The versatility of these new techniques appears in almost all aspects of applied and basic entomology. At the molecular level, coding and replication of DNA have been followed by isotope application. At the cellular level, nuclear radiosensitivity relative to cytoplasmic radioresistance has been determined by irradiating insect eggs. At the tissue and organ levels, the concentration and distribution of tagged insecticides have been determined, thereby contributing to our understanding of the physiological mechanism of toxicology. At the level of the organism itself, the performance and behaviour of tagged individuals, both caged and free-living, have been investigated. Thus our understanding of the abundance and distribution of insect species has been greatly increased.

Isotopes and radiations are becoming increasingly important in entomology, and the dissemination and exchange of information on their manifold uses represent an important factor in the success of pest eradication programmes throughout the world. The International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations jointly convened a Symposium on the Use of Isotopes and Radiation in Entomology, which was held at the IAEA Headquarters in Vienna from 4 to 8 December 1967. This was the third Symposium to be held on this subject; the two previous ones had taken place in Bombay in 1962 and in Athens in 1963.

The range of subjects was inevitably wide owing to the overlapping of various fields of study. The papers are representative of present trends, although only a few papers described applications of radioisotopes in physiology or biochemistry.

The heterogeneity of the information presented at the Symposium was then to be expected. It is by the integration of specialized information, however, that a basis for significant scientific advances is provided. An example of this process is in the successful development of eradication procedures that involve the release of large numbers of insects previously sterilized by radiation: this demands an understanding not only of the basic biology and of radiation biology but also of the technology involved in mass releases at optimal times and in optimal quantities.

The use of radiation in the sterile-insect release method is being applied or considered by many Member States of the IAEA. The method is highly sophisticated. It is neither easy nor a panacea. It is successful only when developed and supervised by competent scientists. The difficulties of applying laboratory results in the field are obvious, especially since the highly selected laboratory organism may differ considerably from the wild type encountered in the field.

The present volume contains the complete proceedings of the Symposium. It is hoped that it will be a valuable source of information to scientists and will stimulate further research in the fascinating and rapidly growing subject of radiation entomology.

EDITORIAL NOTE

The papers and discussions incorporated in the proceedings published by the International Atomic Energy Agency are edited by the Agency's editorial staff to the extent considered necessary for the reader's assistance. The views expressed and the general style adopted remain, however, the responsibility of the named authors or participants.

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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A

ISOTOPE APPLICATIONS: ECOLOGY

(Sessions I and II)

Chairman: H. MARCHART

GHANA

RADIOTRACER STUDY OF THE PREDATORS ON Distantiella theobroma (DISTANT) (HEMIPTERA: MIRIDAE)

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Abstract

RADIOTRACER STUDY OF THE PREDATORS ON Distantiella theobroma (DISTANT) (HEMIPTERA: MIRIDAE). The paper describes a method for labelling the cocoa capsid Distantiella theobroma (Distant) with ^{32}P in sucrose solution. The label was excreted with a biological half-life of six days. When taken up from a labelled natural substrate (cocoa pods) the ^{32}P activity in the insect attained an equilibrium value after nine days and was excreted with the same half-life. Labelled larvae in the field were exposed to the natural predator population, which was sampled by pyrethrum knock-down after 48 hours. None of the predators identified is specialized as a capsid predator. The broad range of predatory species reflects the high species diversity within the tropical forest zone, which is considered to be an important factor in integrated capsid control.

1. INTRODUCTION

Cocoa capsids (Miridae) are pests of major economic importance and a continual threat to West African cocoa production. In Ghana, Distantiella theobroma (Distant) is the most important species. Chemical control of this insect was achieved with lindane, but the problem of resistance to this insecticide and the implications which arise from the continuous use of broad spectrum pesticides necessitated an ecological approach which could eventually lead to more selective methods of control. The present study accordingly deals with the identification of the predators of Distantiella.

A number of predatory insects associated with cocoa capsids was listed by Squire [1], but evidence based on field observation was mentioned only for five species of spiders and three species of mantids, the names of which were not given. Williams [2] observed the ants Pheidole megacephala (F.), one Camponotus species, and Oecophylla longinoda (L.) consuming mirid larvae. He cited indirect evidence that, on seedling cocoa, ants, mantids and reduviids are of about equal importance for natural control of the capsid, but he did not mention which species were contributing. In a recent study, Gerard [3] confirmed predation by Oecophylla longinoda and mentioned one species of Ascalaphidae which feeds on Distantiella in the laboratory.

Prey-predator interrelationships were evaluated with radiotracers by Jenkins et al. [4, 5], Baldwin et al. [6] and James [7] for various species of mosquitoes; Clark [8] detected predators of ^{32}P-labelled pink boll worm moths. The use of the technique for the quantitative determination of herbivore consumption by predacious insects was demonstrated by Crossley [9].

2. EXPERIMENTAL

2.1. Material

Distantiella theobroma was laboratory reared in small numbers by Raw [10] and Prins [11], but an effective method for mass rearing has not yet been developed. This is due to (a) the fragile nature of the mirid, which makes manipulation difficult, and (b) the fact that this sucking insect is a highly destructive feeder requiring large quantities of fresh cocoa tissue daily. Therefore, field-collected 4th and 5th instar larvae were used, which, before labelling, were kept in the laboratory for 24 hours on fresh cocoa shoots at 24°C. There was a daily mortality rate of about 15% of the bugs so treated.

2.2. Labelling of larvae

For predator studies the prey should be labelled internally, since some predators, notably spiders, will not consume the chitinous parts of the prey.

Preliminary experiments showed that labelling from a natural diet, e.g. radioactive cocoa shoots, was relatively inefficient, since only a small fraction of the radiophosphorus applied was translocated into the plant tissues on which the insects feed. Consequently, a technique similar to that used by Lewis and Waloff [12] on the mirid Orthotylus virescens was adopted. The set-up shown in Fig. 1 was devised with the aim of minimizing manipulation of the fragile insect.

The labelling medium was ^{32}P, orthophosphate, with a specific activity of 10-30 μCi/ml in 5% sucrose solution containing 0.1% potassium dihydrogen phosphate as a carrier.

Each 'feeding bell' contained 1 ml solution on a 9-cm (Whatman No. 1) filter paper disc for labelling 25 larvae. The method has the advantage of avoiding the necessity of CO_2 anaesthesia (which adversely affects Distantiella larvae) for handling the labelled insects, as they can conveniently be transferred by inverting the device so that they slide into the transport container. The larvae were kept on the radioactive diet for 16 hours.

2.3. Labelling of cocoa trees

To compare the insects' uptake of ^{32}P from sugar solution with that from a natural substrate, bugs were fed on labelled cocoa trees. Cornwell [13] demonstrated that a uniform distribution of ^{32}P is difficult to obtain by trunk implantation. Therefore, the bark infusion method was used (Fig. 2). This resulted in a sufficiently uniform distribution of activity over the entire tree (5 year-old Amazon), but required a high dosage (10-20 mCi per tree) because the uptake through bark was slow and incomplete even after removal of the dead outer cortex layer.

2.4. Determination of ^{32}P in capsids

To determine the biological half-life of ^{32}P in Distantiella theobroma, the labelled bugs were wet-ashed and counted in a Geiger-Müller liquid

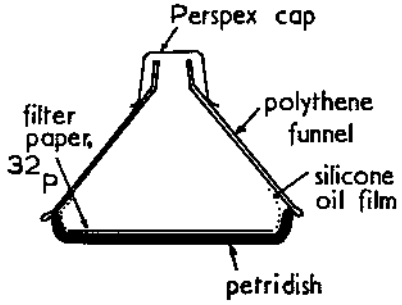

FIG. 1. Set-up for labelling Distantiella larvae.

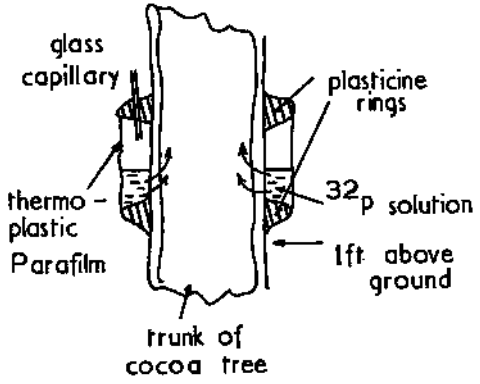

FIG. 2. Bark infusion of ^{32}P into cocoa tree (cross-section).

counter tube. For live counts a whole-body counting cell was used, placed under an end-window counter (1.5 mg/cm^2).

2.5. Release and sampling

Fifty 4th and 5th instar larvae were released on each tree onto a clump of leaves pinned at the main fork. The bugs were allowed to disperse for 48 hours.

Sampling of the entire insect fauna on mature cocoa was done by saturating the canopy with an aqueous pyrethrum emulsion (0.1% active ingredient) at a rate of 1 gal/tree by means of a motor-powered knapsack mistblower. The insects knocked down were collected after 1 hour from cotton sheets spread on the ground under the trees, and the radioactive specimens were sorted out.

3. RESULTS AND DISCUSSION

3.1. Uptake and biological half-life of ^{32}P

Although the feeding conditions in the 'labelling bell' were quite different from those in the field (Distantiella is a phloem feeder), ^{32}P was readily taken up by the insect. The total radioactivity taken up within a

period of 16 hours varied greatly among individuals, the coefficient of variation being 35%.

Larvae that had absorbed 0.25 μCi showed increased mortality when compared with larvae kept on fresh cocoa shoots. It is, however, not clear to what extent this was caused by radiation effect on the one hand and the artificial diet on the other. At a concentration of 0.08 μCi/insect, which was the average level chosen for the field experiments, the mortality was slightly but not significantly increased over that of unlabelled insects.

To establish the period that the larvae would remain labelled, the biological half-life was determined by counting samples from a batch of labelled larvae at various intervals after tagging. From Fig. 3, curve (a), it can be seen that the radiophosphorus was excreted at a rate which gave a half-life of 6.2 days. The scatter of the measurements reflects the variation in the ^{32}P uptake among individual larvae.

To determine how much of the activity of the labelled insects was due to surface contamination, a batch of 10 larvae was kept on a radioactive cocoa pod from a labelled tree and the uptake and excretion of the label measured by counting the live individual insects in the whole-body counter cell.

The ^{32}P level rose rapidly during the first five days of feeding and thereafter levelled off as the excretion balanced the uptake (Fig. 3, curve (b)). After nine days, the insects were transferred to a non-radioactive diet and ^{32}P was excreted with a half-life of 6 days. As this did not differ significantly from the half-life mentioned above, it was concluded that surface contamination was of minor importance with the standard labelling procedure. The biological half-life of 6 days, combined with the physical isotope half-life of 14.3 days, gives an effective half-life of 4.2 days. This means that the label should remain detectable for about three weeks after tagging. In the field, however, dispersal of the insects was the factor limiting duration of the experiments, not the persistence of the label.

Labelling cocoa capsids in the field on tagged trees was impractical for predator studies because of interference by other phytophagous insects (mealybugs, psyllids, leafhoppers) and the relatively low level of activity acquired by the plant tissue.

3.2. Spectrum of predators

The larvae released in the field were exposed to all natural predators present. These could be identified in the knock-down samples by means of the radioactivity they had acquired by feeding on the labelled Distantiella larvae.

The distribution of the radiophosphorus within the knock-down insect fauna in 12 canopy replications in which a total of 3000 Distantiella larvae had been released is shown in Table I.

The possibility of a contribution from the third and higher links of the food chains was neglected. This appeared justified on the basis of size differences between the respective predators, the high ratio of the number of labelled capsids to that of the radioactive predators recovered, and the short time of interaction between prey and predators.

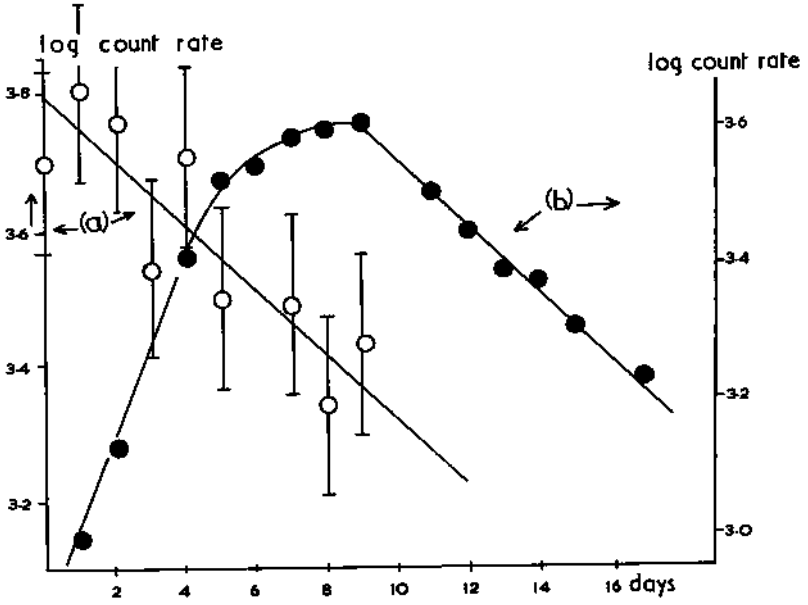

FIG. 8. ^{32}P activity in Distantiella theobroma
Curve (a): excretion after up-take from sucrose solution on filter paper.
Curve (b): Up-take and excretion from labelled cocoa pods.

Some slightly active specimens, other than ants, were presumed surface-contaminated through contact with the labelled larvae in the knock-down samples and are not included.

The trophic position of the radioactive tettigonids (longhorn grasshoppers: Afrogryllacris africana (Brunner), Amyttina quadrimaculata (Karny), Amyttina aliquantula (Karsch) is not known, but as these had acquired distinctly less ^{32}P than other species they were regarded as scavengers. The same applies to the few radioactive specimens of blattids and Diptera. The ^{32}P activity of the ants was usually comparatively low, since the label was distributed over a large number of individuals through food exchange within the colonies.

To distinguish predacious from scavenger ants, observations were made in the field and only species found to attack live capsids on the cocoa tree were classified as predators.

The following species of predators were identified:

Formicidae: Platythyrea conradti Emery
Oecophylla longinoda (Latreille)
Crematogaster depressa (Latreille)
Crematogaster africana (Mayr)
Camponotus 'barbarus'
Macromischoides aculeatus (Mayr)

Reduviidae:	Rhinocoris carmelita (Stal)
	Rhinocoris albopilosus (Signoret)
	Vestula lineaticeps (Signoret)
	Rhinocoris obtusus (P. de Beauvois)
	Endochus africanus Bergroth
Mantodea:	Sphodromantis lineola (Burmeister)
	Cataspilota misana (Giglio-Tos)
	Panurgica compressicollis (Saussure)
	Prohierodula ornatipennis (Bolivar)
	Tarachodes gerstaeckeri (Werner)
Arachnida:	Viciria equestris Simon
	Viciria ocellata (Thorell)
	Hyllus holochalceus Simon
	Telamonia sp. n.
	Chiracanthium sp.

and nine more species, so far unnamed, including a salticid of a new genus and two species which probably belong to Cispius and Apochinomma.

Platythyrea conradti, a large ponerine ant, was hitherto not regarded as preying on capsids [3]. By tracing workers carrying labelled prey with a field counter, the nests of this species were found in the dead wood of half-rotten tree stumps. The colonies contained only up to one hundred individuals. The workers are highly active solitary foragers, and systematically search the entire surface of the cocoa tree. This behaviour might partly compensate for the low population as far as this species' efficiency as a capsid predator is concerned. Since it is a ground nester, this ant is partially protected from insecticides applied to the cocoa tree.

Oecophylla longinoda and the two Crematogaster species are arboreal ants, widespread and occurring in large numbers. They are known to be mutually exclusive species. Oecophylla is the more desirable predator from the viewpoint of the cocoa grower, as it does not attend Pseudococcus njalensis, the most important vector of the Swollen Shoot virus. It is, however, more likely to be affected by spraying, as it nests in the cocoa canopy, whereas Crematogaster mostly nests on high forest trees well beyond the reach of the spray drift.

Macromischoides aculeatus and Camponotus 'barbarus' were occasionally observed attacking capsids on cocoa. They seemed to be of little importance as capsid predators.

The other Camponotus species were not observed preying and were therefore considered solely as scavengers.

The reduviids listed, with the exception of Endochus africanus, were collected by hand from young cocoa. In these plots a continuous canopy had not yet formed, and the abundance of weeds supported a rich fauna characteristic of the herb and shrub level. Endochus africanus was the only radioactive reduviid obtained from forest cocoa.

Out of a total of 78 mantids, 15% were radioactive. This suggested that mantids were the most active predators. Sphodromantis lineola accounted for one third of the radioactive specimens.

Of the spiders, 1.2% of the specimens had fed on capsids. Because of their high population level they were regarded as important in capsid

TABLE I. DISTRIBUTION OF ^{32}P IN KNOCK-DOWN SAMPLES

	Total number of specimens	Number of radioactive specimens
Ants		
Oecophylla longinoda	3410	2848
Camponotus barbarus	505	10
Camponotus spp.	7963	6813
Macromischoides aculeatus	366	134
Crematogaster africana	15	15
Crematogaster depressa	776	634
Platythyrea conradti	33	16
Reduviids	2	1
Spiders	1263	15
Mantids	78	12
Tettigonids	461	13
Blattids	188	4
Diptera	523	2

control. Two thirds of the radioactive specimens belonged to the salticids. As relatively little is known about West African spiders, 9 species could not be named. They were sent to the British Museum for identification.

As regards the impact of predation on the population dynamics of the cocoa capsid in the field, it became clear during the study that the experimental set-up, while being highly efficient for evaluation of the qualitative composition of the predator spectrum, was of limited value for a quantitative interpretation. This was for the following reasons:

The recovery of ^{32}P in the samples was of the order of 10% of the input activity, including recovered labelled capsids. Thus it was clear that the experiment was far from approximating a closed system. The relatively high mortality of the larvae available for the experiments was a complication masking the effect of predation. The release of

50 Distantiella larvae on one tree represented an artificial situation, as it established a high local population density seldom occurring in the field. As predation intensity often is a function of the population density of the prey, this would lead to an unrealistic estimate of the predator activity. The labelled capsids for some time after release were not fully adjusted to their habitat. With an insect which is characterized by its cryptic behaviour, the resulting increased locomotory activity needed in search of shelter and suitable feeding sites was bound to exaggerate the effect of predation.

The relative importance of ants, mantids and reduviids was discussed by Williams [2]. According to his estimates, these three taxa were about equally efficient in capsid control. The results of the present study suggest that the contribution from the reduviids is of minor significance at the canopy level of mature trees. On young trees, where a high light intensity on the ground favours a rich weed flora, reduviids can be important. Predation by spiders – which has been neglected so far – must be considered comparable to that of mantids. The relative importance of ant predation was impossible to estimate because only the worker caste could be sampled, but an unknown part of the prey would end up outside the sampling area in the nests in the ground in adjacent cocoa stands and on high shade trees. There is no indication of any of the named species being specialized or dependent on capsids, as all occurred in samples from non-infested cocoa.

The large number of species involved in capsid predation reflects the general phenomenon of high species diversity characteristic of the tropics, particularly the forest zone. This is of some practical significance for the establishment of an integrated capsid control programme, since the possibility of serious interference of chemical control with natural control by predation will be reduced by a high species diversity among the predators.

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REFERENCES

[1] SQUIRE, F.A., Revta. Ent., Rio de J. 18 (1947) 219.
[2] WILLIAMS, G., Bull. ent. Res. 45 4 (1954) 723.
[3] GERARD, B.M., Half-Yearly Progress Report, Cocoa Res. Inst. Ghana No. 4 (1964) 28.
[4] JENKINS, D.W., HASSET, C.C., Nucleonics 6 (1950) 5.
[5] JENKINS, D.W., KNIGHT, K.L., Proc. ent. Soc. Wash. 52 (1950) 203.
[6] BALDWIN, W.F., JAMES, H.G., WELCH, H.E., Can. Ent. 87 (1955) 350.

[7] JAMES, H.G., Can. J. Zool. 43 (1965) 155.
[8] CLARK, E.W., GLICK, P.A., J. econ. Ent. 54 (1961) 815.
[9] CROSSLEY, D.A., Jr., in Radiation and Radioisotopes Applied to Insects of Agricultural Importance, IAEA, Vienna (1963) 43.
[10] RAW, F., Bull. ent. Res. 50 1 (1959) 11.
[11] PRINS, G., Proc. Mirid Control Conf., Tafo (1963) 33.
[12] LEWIS, C.T., WALOFF, N., Entomologia exp. appl. 7 1 (1964) 15.
[13] CORNWELL, P.B., Trop. Agric. Trin. 34 2 (1957) 132.

APPENDIX

COCOA RESEARCH IN GHANA: THE ROLE OF RADIOISOTOPES IN THE STUDY OF INSECT PESTS

Cocoa farming is the most important single factor in Ghana's economy, and this is reflected by the fact that cocoa accounts for one half to two thirds of Ghana's export earnings. Ghana is the world's largest cocoa producer, and there are approximately 4.5 million acres (1.8 million hectares) under cocoa, yielding over 400 000 tons of raw cocoa annually and averaging 36% of the world's production.

The cocoa farmer faces two major threats from insect pests: capsids (Miridae) and mealybugs (Coccidae). Both affect the tree rather than the crop: capsids destroy the phloem tissue, causing die-back of the tree, whereas the mealybugs spread the Swollen Shoot disease, a virus infection which usually results in the death of the cocoa tree. These two pests have been devastating vast areas of the richest cocoa regions, the capsids alone causing losses estimated at 20-30% of the national yield. In addition, various minor lepidopterous pests, pentatomids, psyllids, thrips and leafhoppers cause further losses. The study of insect pests within the ecosystem of the cocoa farm has therefore been, and still is, a major concern of cocoa research, and radioisotopes are considered an invaluable tool for this work.

Early work by Cornwell was concentrated on the species Pseudococcus njalensis Laing, the dominant vector of the Swollen Shoot virus. Uptake and translocation of ^{32}P was studied in the laboratory and in the field on seedlings and mature Theobroma cacao. Techniques of labelling with ^{32}P by trunk implantation, root immersion and soil application were compared [1].

The practice of controlling Swollen Shoot by cutting out infected trees has to take into account the dispersal pattern of virus-carrying mealybug larvae. Dispersal studies with ^{32}P-labelled Pseudococcus njalensis showed that under Ghana farm conditions larvae can migrate to adjacent trees via contacting branches [2], and better control can therefore be achieved by cutting out apparently healthy trees which are in contact with the diseased trees [3].

In another study, Cornwell [4] worked on the dispersal of labelled mealybugs on the ground, showing that movement from heaps of virus-infected timber to nearby healthy stands constitutes no serious risk of spreading the infestation.

It was also demonstrated that Pseudococcus njalensis is attended by ants of the genus Pheidole [4] and by Crematogaster striatula Emery [1].

The latter species accumulated ^{32}P to a greater extent than its host. Ant-mealybug relationships proved to be of some practical significance in so far as adult mealybugs can be carried to, and established on, adjacent trees by attending ants.

Roivainen [5] worked on some aspects of food exchange in ant colonies of the species Crematogaster striatula with the aim of investigating the possibility of chemical ant control by means of insecticide baits. He demonstrated that ^{32}P applied in a honey bait solution is distributed into all developmental stages of the colonies within four weeks, pupae and young workers being the last stages to acquire the label.

The same author also carried out studies on the feeding behaviour of Pseudococcus njalensis on cocoa seedlings, with the object of obtaining information on virus transmission by this sucking insect. He was able to show that the labelling of virus-infected seedlings with ^{32}P is an effective method of assessing the extent to which mealybugs that have fed on infected plants are infective. Feeding was shown to be a function of temperature.

^{32}P-labelled Distantiella theobroma (Distant), the black cocoa capsid, was used for testing sampling techniques in ecological studies. It was shown that the pyrethrum knockdown technique used at the Cocoa Research Institute for sampling the canopy population of cocoa capsids gives a recovery of approximately 85% of the large larvae and above 90% of the adult insects [6].

The absolute population density of cocoa capsids in infested farms had been estimated hitherto by means of hand collection. This method is liable to be quite inaccurate. By release and recapture of labelled larvae the author found that previous estimates of the maximum level of infestation were low by a factor of at least two [7]. On the basis of these tracer experiments, the sampling procedure has been modified to give a more reliable index of the level of infestation.

To acquire information on the factors involved in natural control of cocoa capsids, a study of the capsid predators among the cocoa entomofauna was carried out. Methods of labelling Distantiella theobroma with ^{32}P were evolved, and by release of tagged larvae in the field it was possible to identify more than twenty species of ants, mantids, spiders and reduviids as capsid predators [8, 9]. Leston [10] studied predation on labelled Distantiella by Oecophylla longinoda and decided that this ant is an important predator. In a colony he found the highest activity associated with the female alates; males contained five times less, and large workers forty times less, ^{32}P than the queens. Small workers had acquired little or no activity.

A study of the dispersal of Distantiella reared on ^{32}P-labelled trees under field conditions gave some information on the flight ability of the insect, but, because of the low recovery of radioactive adults, this was not conclusive [11].

^{137}Cs was effective as a tracer for the sap stream in the cocoa tree during feeding experiments. The very short biological half-life of this isotope in the cocoa capsid (4 hours) suggests that it could be applied for differentiation of short-term from long-term migration by using the tracer as a clock to mark the time of departure from the point of release in dispersal studies [11].

Further work along the following lines would be promising:

Bathycoelia thalassina (Herrich-Schaeffer), a pentatomid which can cause heavy losses to the crop by inhibiting bean development [12], should be the object of dispersal studies. The release-recapture technique will be useful in evaluating sampling methods necessary to determine the population level in infested farms. Such investigations are of the utmost value, since crop damage is virtually invisible in the field and manifests itself only at the pod breaking stage.

The role of ants within the ecosystem of the cocoa farm has been the object of a number of studies in the past, but it is not yet understood in quantitative terms. Large-scale experiments on food chains should give valuable information with regard to the agricultural importance of the species Oecophylla longinoda (Latreille), Crematogaster africana (Mayr) and depressa (Latreille), and Platythyrea conradti Emery as predators on the cocoa capsids Distantiella theobroma and Sahlbergella singularis (Haglund).

The study of food chains will increase understanding of insecticide side effects in heavily sprayed plantations that result in outbreaks of lepidopterous pests [13] such as the cocoa trunk borer (Eulophonotus myrmeleon Fldr.) and the pod husk miner (Marmara ssp.). This work could be incorporated in the large-scale ^{32}P superphosphate experiments being planned by the Cocoa Research Institute in co-operation with the IAEA. The wide species diversity in a tropical environment presents problems which can be solved only by a long-term approach.

The use of labelled baits will provide information on the foraging range of ants, which is needed for predacious species and especially for those attending the vector of the Swollen Shoot virus.

Relatively little is known about pollination of the cocoa flower. The distance over which cross pollination by the ceratopogonid midges Forcipomyia ashantii (Ingram and Macfie), Forcipomyia ingrami (Carter) and Lasiohelea litoraurea (Ingram and Macfie) takes place is an important factor in plantations of self-sterile clones, where compatible pollinator trees have to be provided. Experiments with radioactively labelled pollen have been suggested to clarify this question.

The study of plant-insect relationships by means of radiotracers could yield information on the physiological aspects of hopper burn, which is caused by Empoasca species on young cocoa [14]. Experiments on the feeding behaviour of Planococcus citri (Risso) in comparison with that of Pseudococcus njalensis would be desirable to obtain information on the relative frequency of virus transmission by these two species.

Generally speaking, with insect problems the importance of the multi-factor approach, i.e. of an integrated view, is now appreciated to a greater extent. Concomitantly with this trend of viewing the pest problem against the background of the cocoa ecosystem, a vast number of questions will arise which can be answered only through radioisotope studies. Although it will be some time before the multi-factor system of this tropical environment can be understood in quantitative terms, many problems hitherto inaccessible to experimentation can be tackled by means of tracer techniques.

This brief review of the scope of radioisotopes in the study of cocoa insect problems is presented to demonstrate how much tracer methods

can contribute to progress in agricultural research, which is of vital importance for a developing country like Ghana.

REFERENCES

[1] CORNWELL, P.B., Trop. Agric., Trin. 34 (1957) 117.
[2] CORNWELL, P.B., Bull. ent. Res. 49. (1958) 813.
[3] CORNWELL, P.B., Annual Report W. A. C. R. I. (1953-54).
[4] CORNWELL, P.B., Bull. ent. Res. 47 (1956) 137.
[5] ROIVAINEN, O.H., Rep. Cocoa Res. Inst. Ghana, 1966/67, in the press.
[6] PICKETT, A.D., MARCHART, H., Rep. Cocoa Res. Inst. Ghana, 1965/66, in the press.
[7] MARCHART, H., Q. Rep. Int. Capsid Res. Team 4 (1966).
[8] MARCHART, H., LESTON, D., Rep. Cocoa Res. Inst. Ghana, 1965/66, in the press.
[9] MARCHART, H., "Radiotracer study of the predators on Distantiella theobroma (Distant) (Hemiptera: Miridae)", these Proceedings, SM-102/1.
[10] LESTON, D., Ghana J. Agric., in the press.
[11] MARCHART, H., unpublished.
[12] LODOS, N., Bull. ent. Res. 57 2 (1967) 289.
[13] ENTWISTLE, P.F., in Agriculture and Land Use in Ghana (WILLS, J.B., Ed.) Oxford University Press, London (1962) 342.
[14] PICKETT, A.D., Q. Rep. Int. Capsid Res. Team 1 (1967) 19.

DISCUSSION

W.J. KLOFT: You mention that Distantiella is a phloem feeder. We know that some phloem-feeding insects, such as aphids, mealybugs and others, secrete honeydew. We have also occasionally found honeydew secretion in a Heteropter, the sugar-beet feeding species Piesma quadratum (Tiupidae). Radioactive honeydew secretion, if it occurs, could be a possible source of error in your experiments, as it would be taken up by the ants. I know that you regarded as predators only those ants which were observed directly attacking the capsids, but I should nevertheless be very interested to learn if any honeydew secretion occurs.

H. MARCHART: Distantiella theobroma does not secrete honeydew. I agree, however, that with other insects such as Pseudococcus njalensis, for example, transfer of the label by honeydew secretion could interfere with predator-prey experiments. The trophic position of ants is sometimes difficult to ascertain and has to be established by observation.

D. LINDQUIST: You released 50 labelled insects per tree; could you give an estimate of the 'normal' population per tree? Also, what percentage of the labelled insects released was destroyed by predators during the 48-hour test period?

H. MARCHART: Distantiella occurs only in small numbers; because of this it is always difficult to obtain statistically significant sample sizes in studies of binomics and related problems. A number as low as 100 insects/acre can cause appreciable damage, and 4-5 insects/tree would be a fairly high natural population density. The percentage of capsids killed by predators in my experiment was about 2% in 48 hours. This would indicate that predators are an important factor in capsid mortality; however, for the reasons mentioned in the paper, predation intensity in my experiments was probably higher than under natural conditions.

W.J. LE QUESNE: I understand that artificial feeding is often difficult in the case of Homoptera. I have seen a recent paper suggesting the technique of using a membrane with the fluid under slight pressure. Would you care to comment more on your technique?

H. MARCHART: The difficulty you mention was anticipated, but it turned out that the cocoa capsid feeds on a solution applied to filter paper to an extent sufficient to obtain labelled insects of any desired level of radioactivity. I have also fed them through Parafilm® membranes to determine the amount of liquid diet taken up per day.

E.M. SHUMAKOV: Have radioisotopes been used in Ghana to study the pollination of the cocoa tree by Meleidae?

H. MARCHART: Not yet, but such an investigation is planned for the future.

ETUDE, AU MOYEN DE L'OR RADIOACTIF ^{198}Au, DU RAYON D'ACTION DE COLONIES DE BOURDONS (Bombus sp.) EN VUE DE LA POLLINISATION DES PLANTES CULTIVEES

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Abstract — Résumé

ETUDE, AU MOYEN DE L'OR RADIOACTIF ^{198}Au, DU RAYON D'ACTION DE COLONIES DE BOURDONS (Bombus sp.) EN VUE DE LA POLLINISATION DES PLANTES CULTIVEES. Il est généralement très facile de procéder au marquage d'une colonie au moyen d'un radioisotope en profitant des incessants échanges de nourriture entre les individus. Les bourdons, bien que vivant en colonie, ne procèdent pas à des échanges directs. Cependant, il a pu être mis en évidence qu'il existait de continuels transferts par l'intermédiaire des cellules à miel (trophallaxie secondaire). Dans ces conditions le marquage d'une colonie de bourdons est aussi facile à réaliser que celui d'une ruche d'abeilles. L'or-198 a été utilisé avec succès pour réaliser ces essais qui ont permis d'obtenir des renseignements importants et inédits sur le comportement des butineuses des colonies de bourdons placées à proximité des champs de légumineuses en vue de favoriser la production de graines. L'accoutumance à un nouveau site, la fixation sur une espèce végétale, le rayon d'action, le déterminisme des lignes de vol et des secteurs de butinage ont pu ainsi être étudiés.

USE OF RADIOACTIVE GOLD, ^{198}Au, TO STUDY THE RADIUS OF ACTION OF COLONIES OF BUMBLE-BEES (Bombus sp.) WITH A VIEW TO POLLINATION OF CULTIVATED PLANTS. It is generally very easy to label a colony with a radioisotope, taking advantage of the constant exchange of food between individuals. Bumble-bees, although living in a colony, do not engage in direct exchange, but it has been shown that continual transfer takes place through the honey cells (secondary trophallaxia). In these conditions a bumble-bee colony can be labelled as easily as a hive of bees. Gold-198 was successfully used for the tests, which enabled us to obtain important and unpublished information on the behaviour of workers in bumble-bee colonies situated close to fields of leguminous crops, with a view to enhancing grain production. Questions that have been investigated by this method include the time required to become familiarized to a new site, fixation on a particular species of plant, the radius of action and the determinate character of the flight paths and the honey-gathering sectors.

1. INTRODUCTION

Depuis quelques années les bourdons font l'objet de nombreuses recherches en vue de leur utilisation pour assurer la pollinisation de certaines plantes cultivées, en particulier des Légumineuses. Cependant, nous manquons souvent d'informations de base sur la biologie de ces insectes, ce qui ne facilite pas leur utilisation à l'échelle agronomique.

On peut constater, par exemple, la rareté des données concernant le comportement de butinage qui, pourtant, seraient essentielles.

Pour tenter de commencer à combler cette lacune nous avons entrepris une étude du rayon d'action et des particularités du butinage en effectuant

un marquage des butineuses au moyen des radioisotopes en nous inspirant de travaux analogues effectués sur l'abeille domestique par plusieurs auteurs [1,2].

2. METHODES DE TRAVAIL

2.1. Le marquage

Pour différentes raisons déjà soulignées lors des travaux portant sur l'abeille, nous avons voulu utiliser l'or radioactif, ^{198}Au, qui, par sa période suffisante pour travailler efficacement mais assez courte pour ne pas contaminer le milieu, continue de nous apparaître comme un traceur très approprié à nos recherches.

Rappelons que ^{198}Au est un élément de 2,7 j de période émettant entre autres un rayonnement gamma de 0,411 MeV.

Cet élément peut être livré sous forme de solution colloïdale facilement absorbée par les abeilles ou les bourdons, mélangée avec une solution de sucre ou de miel.

De même qu'en ce qui concerne les abeilles, nous avons estimé qu'une activité moyenne par insecte d'environ 1 μCi permettait une détection facile et limitait l'irradiation des bourdons. L'activité totale de la colonie de bourdons était ainsi au maximum de 2 à 3 mCi dans le cas de colonies d'importance normale.

Un problème se posait cependant, a priori, en ce qui concerne la distribution du radioélément traceur. La question est facilement résolue chez l'abeille domestique, les fourmis, les guêpes et les termites, grâce à la particularité présentée par ces insectes d'échanger continuellement entre eux de la nourriture. Il suffit dans ces conditions que quelques individus reçoivent le traceur pour que l'ensemble de la colonie soit marquée au bout d'un laps de temps très court, et ceci de manière relativement uniforme. Cette particularité a d'ailleurs facilité un grand nombre de travaux sur la sociologie des insectes.

Mais chez les bourdons, qui sont pourtant des insectes sociaux, ce phénomène de transfert de nourriture, généralement connu sous le nom de trophallaxie, semblait ne pas exister.

Il est en effet impossible de constater l'existence d'échanges directs entre individus appartenant à la même colonie. Pourtant il a été possible de démontrer [3] l'existence d'une trophallaxie indirecte d'un type nouveau.

Une seule ouvrière d'une colonie de Bombus hypnorum composée d'environ soixante individus reçut à l'aide d'une pipette une dizaine de millimètres cubes de miel contenant quelques microcuries de ^{198}Au. Cette ouvrière fut relâchée et il fut possible de constater que la nourriture ingérée par un seul individu se diluait avec une très grande rapidité dans la population. Cinquante et un pour cent des bourdons présentaient des traces aisément décelables de radioactivité 6 h seulement après le retour de l'ouvrière.

Différentes observations nous ont ensuite montré qu'il existait bien une trophallaxie indirecte du fait de l'habitude des bourdons de venir à leur retour dégurgiter de la nourriture dans des «pots à miel» qui servent à l'alimentation de l'ensemble de la population. Cette particularité permet

donc d'opérer le marquage par radioélément d'une colonie de bourdons avec autant de facilité que dans le cas des autres insectes sociaux.

Deux techniques peuvent être envisagées: la première consiste à donner la nourriture contenant le traceur à une ou plusieurs ouvrières au moyen d'une pipette ou à l'aide d'un «nourrisseur extérieur», c'est-à-dire d'un petit récipient placé aux environs immédiats de la colonie.

La seconde consiste à introduire dans la colonie un récipient contenant la nourriture marquée. Ce récipient joue le rôle de pot à miel et sert à l'alimentation d'un grand nombre d'individus. La première technique permet de mieux contrôler les quantités de nourriture ingérées, donc les doses reçues par les insectes; la seconde permet d'effectuer plus rapidement un marquage plus global. D'autre part, dans ce dernier cas, le pot à miel artificiel restant en place est continuellement visité et, bien que son activité par unité de volume décroisse en fonction de la période du radioélément, cet apport permet de compenser les effets de la période biologique chez les insectes et d'effectuer la détection dans de meilleures conditions les deuxième et troisième jours de l'expérience.

2.2. La détection

De même que dans nos travaux antérieurs sur les abeilles domestiques, il nous a semblé plus efficace de capturer tous les individus rencontrés dans la zone étudiée avec un filet à insecte afin de mesurer à loisir leur radioactivité à l'aide d'un détecteur portatif, ceci étant lié à notre préoccupation d'utiliser les doses les plus minimes compatibles avec notre expérimentation.

Le détecteur utilisé était un détecteur portatif à scintillation (SPP2). La détection était assurée sur le terrain par deux équipes de deux personnes, l'une capturant les bourdons avec le filet, l'autre pratiquant leur examen au moyen du scintillateur.

3. RESULTATS

Les expérimentations effectuées en 1966 et 1967 ne peuvent encore être considérées que comme des mises au point de la méthode; pourtant elles nous ont déjà apporté des renseignements intéressants que nous allons considérer sans rentrer dans le détail des observations.

Nous avons ainsi pu constater que les bourdons, de même d'ailleurs que les abeilles domestiques, agrandissent graduellement leur rayon d'action après déplacement de la colonie dans un territoire inconnu. Les butineuses n'ont guère tendance à s'écarter beaucoup durant le premier jour et nous n'en voulons pour preuve que les proportions d'individus marqués récoltés dans une population de butineuses dans un rayon d'environ deux cents mètres autour de la colonie.

Le 15 juin 1966, jour d'arrivée de la colonie sur le terrain d'expérience, nous avons capturé six ouvrières marquées sur un total de 168, tandis que le lendemain nous n'en trouvions que une sur un total de 140. Le 22 juin 1967, jour d'arrivée de la colonie, nous capturions six individus marqués sur un total de 75; le lendemain nous n'en trouvions que un sur un total de 141. Pourtant des contrôles nous ont montré que, dans les deux

cas, plus de 95% des butineuses étaient marquées durant toute la durée de l'expérience et qu'elles pouvaient être facilement reconnues.

Un deuxième renseignement est celui relatif à l'effet du microrelief. Plus encore que pour l'abeille domestique les obstacles placés à proximité immédiate de la ruchette contenant la colonie nous semblent jouer un rôle sur la direction choisie par la majorité des butineuses. Ces deux données ont pu être vérifiées aussi bien en ce qui concerne Bombus terrestris que B. Lapidarius.

On a pu également faire quelques observations sur les préférences alimentaires des colonies, la conjonction des deux derniers facteurs entraînant une très grande hétérogénéité de la distribution des ouvrières de la même colonie dans l'espace environnant.

4. CONCLUSION

La méthode de marquage par radioélément semble donc parfaitement applicable à l'étude du butinage des bourdons. La méthode présente d'incontestables avantages par rapport à celle qui consisterait à utiliser des marques colorées, car elle est bien moins longue à mettre en œuvre et ne perturbe pas la colonie. Il semble d'ailleurs particulièrement avantageux de travailler en même temps sur plusieurs colonies d'espèces différentes, ce qui augmente le nombre des reprises et des données utiles pour l'interprétation, sans supplément de travail.

Les premiers résultats obtenus sont encourageants. Ils semblent montrer un grand parallélisme entre ce que nous savons du butinage de l'abeille domestique et celui des bourdons. Pourtant, les précisions que l'on pourra obtenir seront précieuses en vue de l'utilisation de ces insectes pour assurer la pollinisation de certaines plantes cultivées. Il serait souhaitable que ces études soient poursuivies sur un grand nombre d'espèces et dans des biotopes différents; malheureusement, la seule autre tentative effectuée à notre connaissance dans ce sens s'est soldée par un échec, les auteurs [4] n'ayant pu retrouver les bourdons marqués.

REFERENCES

[1] COURTOIS, G., LECOMTE, J., Int. J. appl. Radiat. Isotopes 5 (1959) 265.
[2] LECOMTE, J., Annls Abeille 3 (1960) 317.
[3] LECOMTE, J., C.r. hebd. Séanc. Acad. Sci. 257 (1963) 3664.
[4] DIABOLA, J., TAIMR, L., Acta Soc. ent. Bohem. 62 (1965) 413.

DISCUSSION

H. MARCHART (Chairman): Of all flowers pollinated by insects in your plots, what percentage would you estimate as pollinated by bumble bees?

J. LECOMTE: This varies greatly depending on the crop involved and a number of other factors, mainly climatic. For example, in France

about 100% of red clover pollination (Tetraploid varieties) takes place via bumble bees.

E. HORBER: Have you contemplated, or indeed tried, labelling pollen?

J. LECOMTE: A method has been developed which consists of growing lucerne in a nutrient solution containing ^{32}P. The pollen becomes labelled, and can be detected in neighbouring flowers by autoradiography. The range of dispersal of the pollen seems to be very short, less than 1 metre.

C.A. PELERENTS: How many insects were there in your bumble bee colonies, and were they reared as colonies or assembled from individuals?

J. LECOMTE: The colony size ranged from 150 to 300. They were produced by rearing from a single queen.

M. COHEN: The labelled honey was in the colony for a period of time, and therefore the larvae were both fed on it and exposed to the radiation. Was there any effect on the larvae, and was the number of adults that emerged from them normal?

J. LECOMTE: I made no special study of the effect of radiation on the larvae, but development of the colonies seemed normal.

G. LE MASNE: To what extent was dispersal of the bumble bees modified by transport of the colony from the laboratory to the experimental area?

J. LECOMTE: Honey-gathering activity was modified by transport, and in particular the range of action was reduced during the reorientation period. This interests us, because it would be advantageous to reduce the range of the workers and thus improve pollination. However, we are also performing experiments with colonies which have not been transported.

RADIOISOTOPES IN THE INVESTIGATION OF INTERRELATIONSHIPS BETWEEN APHIDS AND HOST-PLANTS

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Abstract

RADIOISOTOPES IN THE INVESTIGATION OF INTERRELATIONSHIPS BETWEEN APHIDS AND HOST-PLANTS. Activities of the aphid on the host-plant, such as probing, starting of feeding and salivation, can be observed by labelling either host-plant or insect with a radioactive isotope. The course of plant sap uptake, assimilation into the organism, haemolymph circulation, and incorporation into different tissues, especially salivary glands and embryos, were investigated with this method. Artificial feeding of aphids through membranes enabled isotope studies to be performed independently of the host-plant. Studies of excretion via honeydew, saliva and new-born larvae were also carried out. Detailed results are presented on the processes of saliva injection and transport in the plant tissue, which are important from the point of view of phytopathology and virus transmission.

1. INTRODUCTION

Homoptera as plant-sucking insects feed in different tissues of their host-plants. According to Kunkel [1], two main types are distinguished with regard to their physiology of nutrition. The first type takes its food out of conducting tissues and was formerly called 'Phloemsauger' or 'Xylemsauger' [2]; Kunkel introduced the more general term 'systembibitor'. The second type feeds in non-conducting tissues and is called 'Parenchymsauger'. For Sternorrhyncha the term 'localbibitor' is proposed [1].

For a long time the physiology of nutrition and various aspects of insect-host-plant relationships were investigated by means of radioisotopes. This was done mainly with phloemfeeders, especially aphids of the family Aphididae. The Aphididae are most important as plant pests and virus vectors, and such experimental work could elucidate more details of nutritional physiology in other aphid families. Similar experiments were started with Chaetophoridae [3] and 'localbibitors' of different aphid families [4]. This work continues in spite of experimental difficulties.

2. UPTAKE OF FOOD

Isotopically labelled natural or artificial food can be followed during the course of ingestion and digestion by aphids by measuring their radioactivity. A particular advantage of this method is that the uptake of radioactive substances through the stinging and sucking mouth parts minimizes overall contamination [5], since only these parts come into contact

with the labelled material. Radioactive phosphorous was the preferred isotope, because phosphate is abundant in all tissues, well translocated in sieve-tubes, and plays an important role in the metabolism. Most of our experiments were done with aphids sucking on herbaceous plants.

To find their food, aphids must recognize their host-plants and within these the sieve-tubes. Before pentrating deeper areas of the plant tissue, aphids pierce the epidermis and one or two cell layers beyond, a behaviour which is called probing-puncturing. Neither measurement nor the sensitive autoradiographic techniques detected radioactivity inside aphids after such probing [6-8]. Measurements showed that the insects take up food only after stinging when they reach the phloem. The activity then suddenly increases. Aphid activity measurements and histological examinations of stylets and saliva sheaths in plant tissues demonstrated the following three points:

(1) Measurement of the activity of individual aphids showed that the shortest period required to reach a sieve-tube was about seven to ten minutes [6, 9, 10].
(2) Group activity measurement showed that only about 10% needed this minimum time. The speed of tissue penetration up to the depth of the sieve-tubes varied from 5 to 30 minutes for single aphids [4, 10, 11].
(3) A certain percentage of aphids failed to reach a sieve-tube, a phenomenon which depends on the anatomical data of a plant species and its different parts [6, 7, 8, 10]. The failure to puncture a sieve-tube was called 'Fehlstich'. For instance, out of a group of Megoura viciae sucking on the stem of Vicia faba only 61% reach the phloem in one hour and all aphids after 5 hours [7]. A more detailed analysis including the influence of temperature on these complex problems is in progress [4].

Another problem is the recognition of suitable host-plants by aphids. Several mechanisms seem to be involved, for example the gustatory discrimination by the epipharyngeal organ [7]. This was shown by the fact that M. viciae [8] and Aphis sambuci [11] take up radioactivity out of Allium schoenoprasum and other non-host-plants at rates corresponding with initial uptake in suitable host-plants. After this initial probe aphids try to leave the non-host-plant. After puncturing the sieve-tube, the aphids registered a rapid increase of radioactivity. During the first three to five hours', uptake (measured as counts/min) was linear with time, later on it became exponential. After about 24 hours the activity did not increase, even when feeding continued. A phosphate saturation had apparently been reached. Uptake and excretion seemed to be equal [6, 7]. A similar uptake was found for the aestivating larvae of Chaetophoria xanthomelas Koch on maple leaves. The latter result, demonstrated with tracer methods, was contrary to the prevailing knowledge that these larvae were not inactive but ingested food [3].

Ingestion of artificial diets was investigated by means of ^{32}P, ^{131}I and ^{86}Rb with Aphis fabae [12-14]. This method provided a known diet by separating these parasitic insects from their natural host-organism with its undefined food supply. Furthermore, it was possible to introduce into aphids all water soluble substances which were not translocated in plants. One application of this method is the following: it was striking that aphids could be reared over 11 generations on a totally synthetic diet containing

no sterols. After addition of sodium-^{14}C-acetate to the diet, labelled cholesterol could be detected in normal aphids with symbiotes, but not in aphids free of symbiotes [15]. Following the method introduced by Mittler, we used the so-called sachets: a nutrient solution is enclosed between two stretched membranes of Parafilm ® and evaporation and contamination are thus prevented. The influence of ecological factors such as temperature, air humidity, light and day period on the food uptake of Aphis fabae was investigated [16].

3. ASSIMILATION AND DISTRIBUTION IN M. viciae

The first detection of radioactivity in the haemolymph of M. viciae occurred 30 minutes after the beginning of food uptake. The ^{32}P level increased linearly up to 10 hours. An analysis of the phosphate fraction primarily showed a high level of inorganic phosphate. After transfer of aphids to non-radioactive plants, the organic part (ATP, ADP, G-1-P, G-6-P, F-6-P, F-1, 6-P)[1] increased [7]. Due to the rapid passage of the tracer into the haemolymph, the different tissues and organs of the aphids quickly became radioactive. Thus the embryos of artificially fed A. fabae had a high activity only one hour after ingestion began [17]. The tracer was incorporated into almost fully developed embryos, e.g. M. viciae fed on labelled V. faba: the first radioactive larvae appeared six hours after feeding. The uptake of the tracer into the salivary glands was of special interest with regard to salivation and is described in a separate section. Radioiodine seemed to have special affinity for the cuticle [14].

4. EXCRETION AND SALIVATION

Elimination of radioisotopes by the aphid is possible through salivation, production of larvae, moulting, and excretion of honeydew. The biological half-life is determined by the course and velocity of all these processes, and varies from about 30 to 50 hours depending on the stage [5]. These data indicated an extremely high metabolism compared with results for terrestrial Heteroptera, which showed half-lives of between 6 and 40 days [18].

The first radioactivity in the honeydew of aphids sucking on labelled plants appeared not earlier than 2.5 hours after the start of uptake [5]. The activity of the honeydew reached a maximum value after about 20 hours, reflecting a time lapse of 20 to 24 hours needed to reach maximum activity. The count rate of honeydew produced by M. viciae on V. faba amounted to about 3% and 1% per droplet of the aphids activity after 4 and 24 hours, respectively. The corresponding data for aesti-

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[1] ATP = adenosine-tri-phosphate
ADP = adenosine-di-phosphate
G-1-P = glucose-1-phosphate
G-6-P = glucose-6-phosphate
F-6-P = fructose-6-phosphate
F-1, 6-P = fructose-1, 6-phosphate

vating larvae of Chaetophoria xanthomelas were about 70 to 25%, suggesting that these larvae assimilate less phosphate than other phloem-sucking aphids which develop without diapause [3].

Aphid saliva is important in the transmission of circulative plant viruses because it might provide a mechanism for the introduction of the virus into the sieve-tubes. According to studies by Kloft and Kunkel [10] on Myzus ascalonicus and by Ehrhardt [7] on M. viciae, ^{32}P was first excreted in the saliva five hours after the start of feeding. The insects were transferred to a non-radioactive test plant after a relatively short feeding time, and the very low activities in the injected saliva were checked by autoradiography. These experiments might serve as a model for determining circulation times of certain plant viruses.

Phosphorus-32 is secreted along with saliva only during piercing and retraction, as was found in experiments with M. ascalonicus [9]. The injected activity did not depend on the duration of feeding; however, ^{86}Rb was secreted continuously by A. fabae feeding on V. faba leaves [13]. This phenomenon was brought about by the fluid phase of saliva secretion [19]. Through salivation, 1.42‰/hour of the original activity of the aphid was transferred into the leaf. If it was assumed that the radioactivity was uniform throughout an aphid weighing 0.5 mg, the results indicated that saliva was injected into the plant at a rate of about 0.7 μg/hour. This is equivalent to 0.7×10^{-3} μl/hour. An average value of about 3.7×10^{-4} μl was found, suggesting that the additional fluid saliva was probably injected more or less continuously during feeding [13]. Labelling of aphid saliva was possible by application of ^{131}I via the plant or an artificial diet [14]. Other types of plant-sucking arthropods such as thrips [20] and spider mites [21] also injected saliva during feeding, as was demonstrated by the use of ^{32}P.

5. DISTRIBUTION AND EFFECTS OF SALIVA

With autoradiographic methods it was shown that ^{32}P-labelled saliva injected by M. ascalonicus was distributed in Viola tricolor leaves. The spreading of saliva depended on the duration of sucking time and mainly followed the veins [9]. The presence of radioactive material in leaf veins adjacent to feeding punctures made by ^{86}Rb-labelled A. fabae was demonstrated [13] (Fig. 1).

Aphid feeding directly affected the physiological processes of the host-plant, i. e. photosynthesis, respiration and transpiration. Saliva seemed to be the most influential factor here [9]. A plant labelled with tritiated water [23] showed changed transpiration after being pierced by an aphid. The phosphate metabolism of plant parts influenced by the saliva of sucking insects was investigated with tracer methods. Uptake of ^{32}P in damaged areas of Picea needles caused by the strongly phytotoxic saliva of the aphid, Elatobium abietinum, was less than that in healthy parts [22]. The distribution of ^{32}P in a leaf with a pouch gall of Tetraneura ulmi differed from that in a normal leaf of the same shoot. The gall itself had a gradient, with a high level of radioactivity around the laceration. It was concluded that the accumulation or exchange of phosphate varied with the phytotoxaemia induced by the saliva of aphids. If the parasite influenced a differentiated tissue, the exchange of phosphate

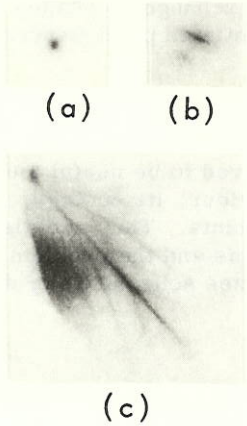

FIG. 1. An autoradiographic presentation of Vicia faba leaves showing the distribution of radioactivity injected by Aphis fabae Scop. which had ingested ^{86}Rb: (a) after 10 min (b) after 1 h, and (c) after 24 h of feeding.

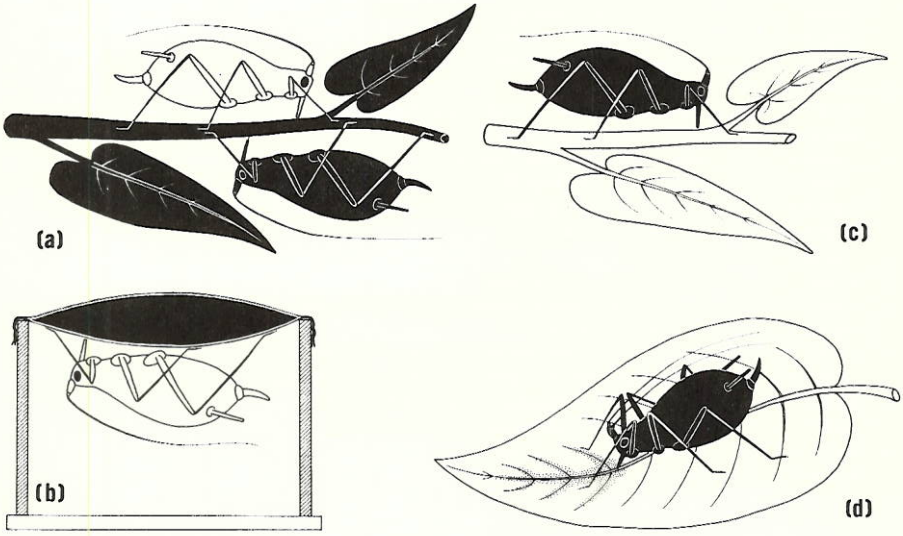

FIG. 2. Schematic summary of radioisotope methods used:
(a) Aphids (white), transferred to a radioactive plant (black), ingest the radioactive food and become radioactive (black).
(b) Feeding aphids with an artificial diet: the radioactively labelled diet (black) is enclosed between two membranes of Parafilm (R), which are stretched over a glass ring to form an aphid cage.
(c) Labelled aphid (black) is transferred to a non-radioactive plant (white).
(d) Radioactive aphid injects labelled saliva which disperses in the leaf (dotted area) primarily along the veins.

decreased. However, the exchange increased with earlier and stronger attacks on normally differentiated plant tissues [24, 25].

6. CONCLUSIONS

Radioisotopes have proved to be useful tools for studying various aspects of the aphid's behaviour, its ecology, nutritional physiology and relationship with its host-plants. They are also valuable in investigations of phytopathological problems and the problem of virus transmission by aphids. Figure 2 summarizes schematically some of the radioisotope methods used.

REFERENCES

[1] KUNKEL, H., Z. angew. Zool. 54 (1967) 37-68.
[2] KLOFT, W., Entomophaga 5 (1960) 43-54.
[3] EHRHARDT, P., Z. angew. Zool. 54 (1967) 21-36.
[4] KUNKEL, H., unpublished.
[5] KLOFT, W., EHRHARDT, P., in Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 181-90.
[6] EHRHARDT, P., Experientia 17 (1961) 461.
[7] EHRHARDT, P., Z. Morph. Ökol. Tiere 52 (1963) 597-677.
[8] EHRHARDT, P., Experientia 19 (1963) 204.
[9] KLOFT, W., Habil. Arbeit, Würzburg 1956; Z. angew. Entomol. 45 (1960) 337-81 and 46 (1960) 42-70.
[10] KLOFT, W., KUNKEL, H., Verhdl. 11. Int. Kongr. Entomol. Wien 1960 1 (1961) 746-49.
[11] KUNKEL, H., Doctoral thesis, Bonn (1966) 172.
[12] DANNEEL, I., Z. angew. Zool. 54 (1967) 181-82.
[13] LAMB, K.P., EHRHARDT, P., MOERICKE, V., Nature, Lond. 214 (1967) 602.
[14] EHRHARDT, P., LAMB, K.P., Int. J. appl. Radiat. Isotopes 18 (1967) 543-44.
[15] EHRHARDT, P., Experientia, in the press.
[16] DANNEEL, I., unpublished.
[17] DANNEEL, I., Z. angew. Zool., in the press.
[18] SCHLAGBAUER, A., Int. J. appl. Radiat. Isotopes 18 (1967) 595-603.
[19] MILES, P.W., Nature 183 (1959) 756.
[20] KLOFT, W., EHRHARDT, P., Naturwissenschaften 46 (1959) 586-87.
[21] LIESERING, R., Z. Pflanzenkrankheiten (Pflanzenpathol.) und Pflanzenschutz 67 (1960) 524-42.
[22] KLOFT, W., EHRHARDT, P., Phytopathol. Z. 35 (1959) 401-10.
[23] KLOFT, W., SCHULZE, E.-F., Atompraxis 13 (1967) 265-69.
[24] KUNKEL, H., Z. angew. Zool., in the press.
[25] KUNKEL, H., Phytopathol. Z., in the ptess.

DISCUSSION

R. CAVALLORO: Were the Aphididae colonies you studied of mixed composition, containing certain proportions of sexual forms, wingless parthenogenetic females and pupae, or did they consist solely of insects at one stage of development? This seems to me important since, from a radioisotope study on trophic relationships between Myzus persicae Sulzer and the host plant Nicotiana tabacum Samsun which I have been conducting since 1961, I have found that the feeding behaviour of the insect varies depending on what stage it is in.

I should also welcome details of the artificial nutritive medium you used, in particular on its pH and its effect on endosymbionts of the aphids.

Finally, I should like to comment on ^{131}I distribution in insects. My work at the Euratom research centre at Ispra has shown that this isotope concentrates in considerable quantities in the cuticle, as well as in the fat bodies. These studies covered a large number of species, including the Orthoptera Conocephalidae, Cryllidae and Gryllotalpidae, the Lepidoptera Noctuidae, Thaumetopoeidae, Saturniidae, Lasiocampidae, Papiliomidae and Pieridae and the Hymenoptera Argidae.

W.J. KLOFT: Our normal basis for any comparison is the behaviour, food uptake, honeydew secretion, etc. of adult wingless parthenogenetic females; up to now most of our work has been with this form. In absolute terms of course, the rate of food uptake by larvae is lower, but in relation to body weight it is higher. This is also true of honeydew secretion (see Mittler's work on Tuberolachmus and P. Ehrhardt's on Megoura viciae). At present we have little knowledge of the food uptake of winged forms; the only study we have made with Aphis fabae was to determine the last food uptake of nymphs before, and the first of the adult after, moulting to the winged stage. This is important in the context of virus vectoring by the winged form.

The pH values of the medium were close to neutral, but slightly alkaline (pH 7.2-7.4). I do not know about any effect of pH variations on endosymbionts; for information on this point you should contact my colleague, P. Ehrhardt.

To your comment on ^{131}I concentration, I would add that results have also been obtained by my colleague, A. Schlagbauer (Ph. D. Thesis, Bonn, 1967), on the storage of ^{131}I in the cuticle of various Heteroptera. The isotope is bound in the lipoproteins of the cuticle, and after tanning this becomes irreversible (see also various papers by Mme. Fuzeau-Braesch).

Rachel GALUN: Mittler found that the phagostimulants of aphids are amino acids and sucrose, which are present in all plants. How, therefore, do the ephipharyngeal receptors differentiate acceptable and unacceptable plants?

W.J. KLOFT: Apart from the substances you mention, mineral salts also have a gustatory effect, as Ehrhardt has shown. However, all these substances are common phloem sap compounds which can be found in plants of different species and even genera or families. The most important factor in distinguishing host-plants from non-host-plants seems to be the existence of secondary plant substances, present in very small quantities and as yet virtually unknown. E. Wensler has found, for example, that the cabbage aphid, Brevicoryne brassicae, is attracted by mustard oils, especially sinigrin. In this case, however, another sensory organ is involved; this is situated on the tip of the labium and allows discrimination from outside.

Rachel GALUN: How did you differentiate between plant and aphid transpiration?

W.J. KLOFT: The effects of feeding on plant transpiration can be detected 5-10 minutes after puncturing by the aphids has begun. As we mention in the paper, uptake of phloem sap can begin at the earliest 8-10 minutes after puncturing; however, for an aphid to ingest, absorb, and begin transpiring tritiated water needs at least 30 minutes. As we

remove our aphids from the plant after a short time, no difficulties arise in distinguishing the two types of transpiration. A much more important source of error would be the secretion of tritiated water with the honeydew, which begins only 2-2½ hours after the start of feeding.

Rachel GALUN: Is it not possible that the increased transpiration rate of the plants is due simply to wounding, rather than to any specific effect of the aphids?

W.J. KLOFT: I have dealt with this aspect in Z. angew. Entomol. 45 (1960) 337-81. The wounding effect is indeed present, but more important are the free amino acids in the aphids' saliva. Their amount and spectrum are closely related to the phytotoxic effect of aphids on transpiration, photosynthesis and respiration of plant tissues (Kloft and Ehrhardt, Phytopathol. Z. 35 (1959) 401).

W.J. LE QUESNE: You mention in your paper that sodium acetate labelled with carbon-14 is converted into cholesterol if endosymbionts are present. Is it also possible that either the insect or the endosymbiont could convert plant steroids, such as sitosterol or stigmasterol, into animal steroids, such as cholesterol?

W.J. KLOFT: This might be possible, but we have not yet started experiments with phytosterols. As we have been able to show, artificial feeding of aphids opens up a wide range of experimental studies on biosynthesis in insects.

K.N. MEHROTRA: Since this effect was very clearly demonstrated in the case of phytophagous insects, especially locusts, I would think that similar phenomena must also take place in aphids.

F.T. PHILLIPS: What range of wind speeds was used in the transpiration experiments with tritiated water?

W.J. KLOFT: We had wind speeds ranging from 0 to approximately 0.5 m/s (1 mph). The wind speed can be changed, but is always precisely controlled and measured with a special flow speed meter.

H. MARCHART (Chairman): I take it that the amount of H_2O vapour lost by evaporation from the insects which you introduced into the test chamber for the transpiration studies was small compared with the water loss from the leaves, so that there was no change in the rate of leaf transpiration caused by alteration of the ambient humidity.

W.J. KLOFT: The water loss of the aphids is indeed extremely low if compared with the leaf's transpiration.

H. MARCHART: Have you considered the application of your methods to the study of systemic anti-feeding compounds?

W.J. KLOFT: Not specifically. We are at present performing a detailed analysis of all our food uptake data, with a view to finding the artificial nutrient medium which is most attractive to the insects. We have started work with systemic insecticides, and are studying their effect on food uptake by measuring the activity of the aphids. The specific activity of the solutions used is always exactly the same. An advantage of our technique is that we do not come up against the problem of metabolism in the plant – we are working with an artificial system which has no metabolism, so that the insecticide compound and the metabolites formed can be checked for both insecticidal and anti-feeding effects. Miss Danneel, who is mentioned in the references to our paper, is working on this.

RADIOISOTOPES IN STUDIES ON THE ECOLOGY OF TICK VECTORS OF DISEASE*

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Abstract

RADIOISOTOPES IN STUDIES ON THE ECOLOGY OF TICK VECTORS OF DISEASE. This paper demonstrates the feasibility of mass rearing of radioisotope-tagged immature ticks by collecting the progeny of engorged females of three species inoculated with carbon-14 glucose or glycine prior to oviposition. The incorporation of radiochemicals internally into ticks reduces the chance of loss of the radioactive label when moulting occurs, or under natural conditions.

Most treated ticks laid eggs. The amount of radioactivity in the progeny could be controlled by controlling the size of the dose administered to the parent ticks. However, differences in radioactivity in the progeny of treated ticks in relation to the day of oviposition were noted. This activity declined progressively through the ninth day. Differences were also noted in relation to the radiochemical used. Most of the carbon-14 glycine (79.0%) received by the engorged females remained in the parents, whereas most of the carbon-14 glucose (77.5%) received was transferred to the progeny. Hatching of eggs labelled by this method was less than in untreated oviposits. Radiosensitivity in the eggs was also noted and was related to the size of the dose administered to the parent tick. No hatching occurred when the average radioactivity of the labelled eggs exceeded 637 counts/min per egg over background. Nevertheless, many highly radioactive eggs hatched, and larvae with counts as high as 510 counts/min per larva over background were observed. The biological characteristics of the tagged larvae were apparently unaffected by incorporation of radiochemicals into these individuals. The proportion of marked larvae which attached to hosts was similar to the proportion of unmarked larvae which attached. The duration of survival of fasting, radioisotope-tagged larvae, under laboratory conditions, was similar to the period of survival of non-radioactive larvae. No apparent loss in radioactivity in fasting larvae held for up to 70 days under laboratory conditions was detected.

This demonstration of the feasibility of mass rearing and long-term survival of radioisotope-tagged immature ticks suggests that it is now possible to apply this radioecological technique to obtain important new knowledge on the ecology of tick vectors of disease.

1. INTRODUCTION

The application of radioisotope labelling methods to studies with ticks was considered by several workers, notably Knapp et al. [1], Quan et al. [2], Hyland and Hammer [3], Babenko [4] and Samostreiskii and Daiter [5]. More recently, Sonenshine and Yunker [6] demonstrated the feasibility of mass production of radioisotope-tagged immature ticks by inoculation of engorged females prior to egg laying, while Sonenshine and Clark [7] showed that it was possible to recapture these immatures.

To realize fully the potential usefulness of the radioisotope tagging method, it is necessary to demonstrate the feasibility of mass production of adequately marked individuals of the vector species, at low cost, and with a label that may be regarded as more or less permanent for that stadium. It is also essential that the biological characteristics of labelled

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individuals, e. g. host-seeking behaviour, survival in the natural environment and related attributes, be similar to those of non-labelled ticks. Finally, it must be demonstrated that the tagged specimens released in a natural area may be recaptured in adequate numbers and readily identified.

This paper describes the laboratory studies which were performed to determine the feasibility of applying radioisotope tagging methods to the study of the ecology of ticks. More specifically, it describes the results of laboratory studies on the efficiency of the labelling method, radiosensitivity, hatching, transtadial transmission, and a comparison of the biological characteristics of marked and unmarked populations. Field trials to determine the usefulness of the technique under natural conditions are described elsewhere [7].

2. MATERIALS AND METHODS

Three species of ticks were used in these investigations, namely Dermacentor variabilis (Say), Dermacentor andersoni Stiles, and Amblyomma americanum (Linnaeus). Ticks were held in an incubator at 80 ± 2°F and 95% r.h., during egg laying, hatching, or moulting periods. Radioactivity measurements were done with a Nuclear Chicago Model 470 gas flow detector and Model 8166 decade scaler.

Inoculations were made according to the method described by Sonenshine and Yunker [6]. Engorged female ticks were positioned on a binocular stereoscopic microscope stage with the aid of plasticine, and this assembly was placed adajcent to a micromanipulator. A 50-μl Hamilton syringe with a 30-gauge needle, containing the desired volume of solution, was fitted onto the micromanipulator and the micrometer controls were used to guide the needle into the body of the tick. The solutions inoculated in the earlier phase of this study were not osmotically balanced; those inoculated later in the study were balanced by the addition of saline to approximate a concentration of 1. 0% NaCl.

Eggs collected for radioactivity determinations were selected at random and segregated into samples of 25, and counts for each sample were recorded for 3-minute periods. Three samples of 25 eggs each were drawn from each oviposit. Oviposits were collected from egg-laying ticks on days 1, 5, 9 and 13 of the oviposition period and checked for radioactivity as described above.

The total number of eggs in an oviposit was determined by observing the weight of a known number of randomly selected eggs, to determine the average weight per egg, and then weighing the entire egg mass. The total number of eggs in the mass was computed from these two figures. Weight determinations were made with a Mettler microgram balance. The proportion of eggs which hatched was determined by counting unhatched eggs.

The efficiency of the radiolabelling process was evaluated. The accuracy of the radioactivity measurements reported for the commercial radioactive preparations used in this study was determined by precipitating 100 lambda aliquots, appropriately diluted on greased planchets, and following each transfer with two rinses of the lambda pipettes with distilled water, and drying slowly under an infra-red lamp. Three samples

were prepared from each of two commercial preparations reported to contain 50 μCi of ^{14}C-labelled glycine. Following the assay of the commercial preparations, 100 lambda aliquots (undiluted) of the same assayed solutions were withdrawn and added to the dissolved, neutralized body contents of each of three engorged female ticks which had not received any radioisotope. Samples were then withdrawn from each of these new preparations, diluted and checked for radioactivity in the same manner as above. In addition, ticks receiving inoculations of known concentrations of radiochemicals were dissolved in acid prior to oviposition, diluted and neutralized, and aliquots were precipitated onto planchets. Finally, the entire oviposits of inoculated egg-laying parents were collected, dissolved and neutralized, and aliquots were withdrawn for comparison of this mode of radioactivity measurement with the measurements made using entire eggs.

Changes in radioactivity in tick material during the processes of (1) hatching, and (2) moulting from larva to nymph were studied by determining the radioactivity of each egg, engorged larva, subsequent stadium and shed material.

Comparison of basic biological characteristics of radiolabelled ticks with untreated ticks was made with D. variabilis. The phenomena studied included (1) willingness to feed, (2) longevity and (3) retention of radioactivity. Fourteen groups of 50 unfed larvae, each seven days old and held at 95% r.h. and 80 ± 2°F, with increasing daily increments of incandescent illumination, were exposed to albino rats. One half of the sample groups were drawn from the second-through-fourth day radioisotope-tagged progeny of a single female. The remaining ticks were non-radioactive individuals drawn at random from untreated parents. All larvae were confined separately, 50/rat, by means of muslin cloth around each animal. Each animal was held over water in a separate tray. The cloth was removed after 24 hours. Engorged larvae were collected and the number of ticks recovered was recorded. Longevity was studied by collecting samples of 100 larvae from both labelled and non-labelled populations immediately after hatching. The ticks were observed each day for evidence of mortality until all were dead. Retention of radioactivity was studied by holding stock populations of 100 larvae and withdrawing 10 individuals at 2-week intervals, killing them with chloroform and determining their radioactivity. This procedure was necessary since anaesthetic techniques were unreliable and care had to be taken to avoid contamination of the detector system by escaping larvae. These studies were continued for 70 days.

3. RESULTS

Mass production of radiolabelled larvae and nymphs of three species by inoculation of preovipositing female ticks was accomplished. Information was obtained on the following aspects: the relationship between the activity of the dosing solution and radioactivity in the progeny of treated parents; the decline in radioactivation of progeny during oviposition; radiosensitivity; differences in radioactivity in immatures in relation to the radiochemical used; the total number of eggs produced and the

per cent hatching; loss of activity with hatching, feeding and moulting; biological characteristics of tagged ticks.

Inoculations. All but two of the 32 engorged female D. variabilis inoculated with buffered saline solutions containing from 12.5 to 25.0 μCi ^{14}C-glucose laid eggs. All ovipositing treated parents transovarially transmitted large amounts of radiochemical to their progeny. The average radioactivity detected in day 1 oviposits ranged from 55.8 counts/min per egg to 976.9 counts/min per egg, with a mean activity of 393.6 counts/min per egg.

Radioactivity of progeny in relation to dose. The transfer of radioisotopic material to the progeny of treated ticks was studied by determining the radioactivity of randomly selected samples of their eggs and larvae. The results of these determinations for D. variabilis are shown in Fig. 1.

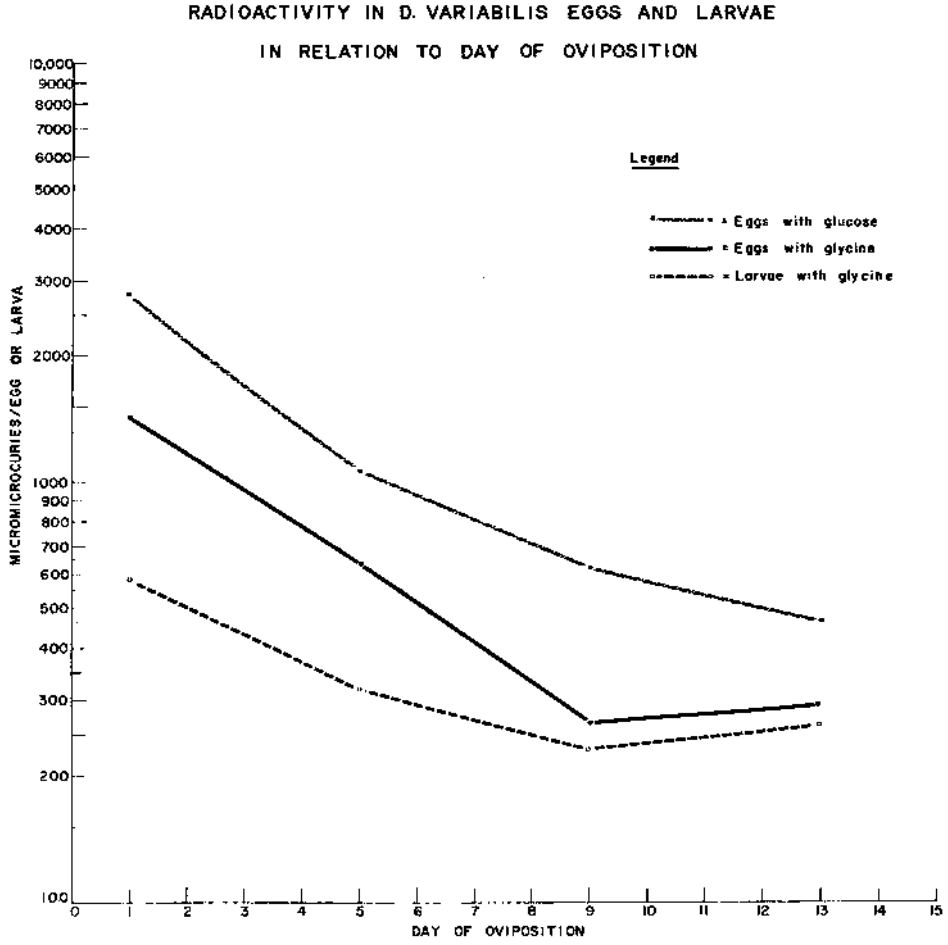

FIG. 1. Graphs showing changes in radioactivity in eggs and larvae from carbon-14 treated D. variabilis females in relation to day of oviposition. Data for larvae from glucose ^{14}C-treated parents was not available. All estimates corrected for difference in efficiency between direct counting (radioactivity) and counting from dissolved material.

These values must be regarded as relative estimates, since measurement of radioactivity in this mode (whole eggs) was inefficient as compared with precipitates from dissolved tick material. The incorporation of radiochemical into eggs and larvae showed a relationship (1) with the period of oviposition, (2) with the activity of the radiochemical solution inoculated into the parent, and (3) with the radiochemical used. The variation in activity in the progeny in relation to the period of the oviposition process in which the eggs were deposited was determined by collecting the oviposits of days 1, 5, 9, and 13. Figure 1 shows the activity present in egg samples at these four different time periods, based upon identical size samples from 33 D. variabilis females inoculated with ^{14}C-labelled glycine and 30 D. variabilis inoculated with ^{14}C-labelled glucose. The radioactivity of the glycine inoculations ranged from 10.5 to 60.0 μCi per tick, and that of the glucose inoculations from 12.5 to 25.0 μCi. In the glycine treated ticks, the average activity per egg was greatest on the first day of oviposition, and this activity declined regularly until the ninth day. Thereafter, the change in activity was very slight, and the average activity per egg on day 13 was slightly higher than on day 9 of the oviposition period. The same trend is apparent in the glucose treated ticks, but the decline in activity was more rapid between the first and the fifth day than was observed in the case of the glycine inoculations.

To compare the relationship between the average activity incorporated into the eggs and the size of the dose administered to each tick, doses of 10.5 μCi, 25.0 μCi, 50 μCi, and 60 μCi of ^{14}C glycine, and 6.5 μCi, 12.5 μCi and 25.0 μCi of ^{14}C glucose were administered to the engorged females. Each inoculation at a given dose level was replicated from six to seven times. The transfer of radioactivity from parent to egg mass in relation to these graded dosages is shown in Figs 2 and 3. Eggs obtained from parents inoculated with large doses generally had higher activity measurements than those obtained from parents inoculated with smaller doses. This is most evident in the case of the glucose treated ticks.

Similar studies were done with D. andersoni and A. americanum. Most inoculated females of both species laid eggs and radioactivity was readily detected in the progeny, at least through the larval stage.

Total number of eggs produced. The mean weight of 18 engorged D. variabilis females taken immediately after dropping from their hosts was 639.1 mg. The average weight of the egg masses produced by 25 ticks, where it was possible to collect and weigh the entire oviposit, was 237.3 mg. The average number of eggs produced by 48 ovipositing inoculated females was 4002. Thus, the parent tick converts approximately 37.1% of the engorged body weight into eggs. Large egg masses were also produced by inoculated D. andersoni and A. americanum females. The average number of eggs produced by the treated D. andersoni was 6632 and that produced by treated A. americanum females was 4463.

Hatching (radiosensitivity). Larvae hatching from radiolabelled oviposits were less radioactive than the eggs from which they hatched (Fig. 1). The average reduction in radioactivity was greatest for the day 1 oviposits, less for the day 5 oviposits, and least in the later day oviposition periods. The average drop in radioactivity represented a loss of 55% from that present in the egg mass. Further, high activity levels were completely lethal to the eggs. Although oviposits with

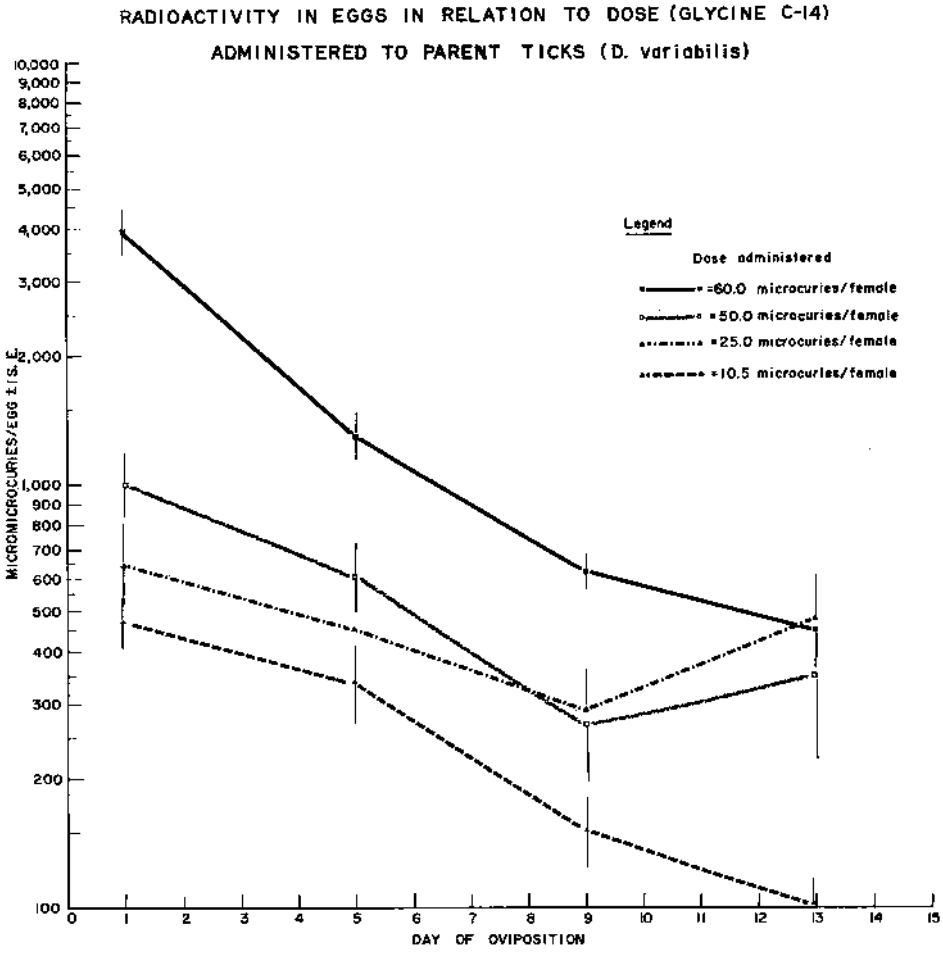

FIG. 2. Graphs showing relative differences in radioactivity in eggs from glycine ^{14}C-treated D. variabilis parents in relation to dose used. Each point represents average of three samples from oviposits of each of 6 to 7 female ticks inoculated with the same dose. All estimates corrected for difference in efficiency between direct counting (radioactivity) and counting from dissolved material.

activities of up to 976 counts/min were obtained, no hatching was observed. In the case of three females inoculated with 60 μCi of ^{14}C glycine and three with 25 μCi of ^{14}C glucose, day 1 oviposits with activities of 757.8 counts/min per egg, 636.8 counts/min per egg, 686.0 counts/min per egg, 933.0 counts/min per egg, 887.8 counts/min per egg, and 976.9 counts/min per egg, respectively, were obtained. However, no hatching occurred in any of the eggs produced by these female ticks. Hatching was considerable at lower activity levels, in many cases exceeding 50%. The relationship between the mean radioactivity of the entire egg mass and the per cent hatching which occurred in the progeny of the ^{14}C-treated parents (glycine and glucose) is shown in Table I. Note that the overall per cent of hatching was 50.4%.

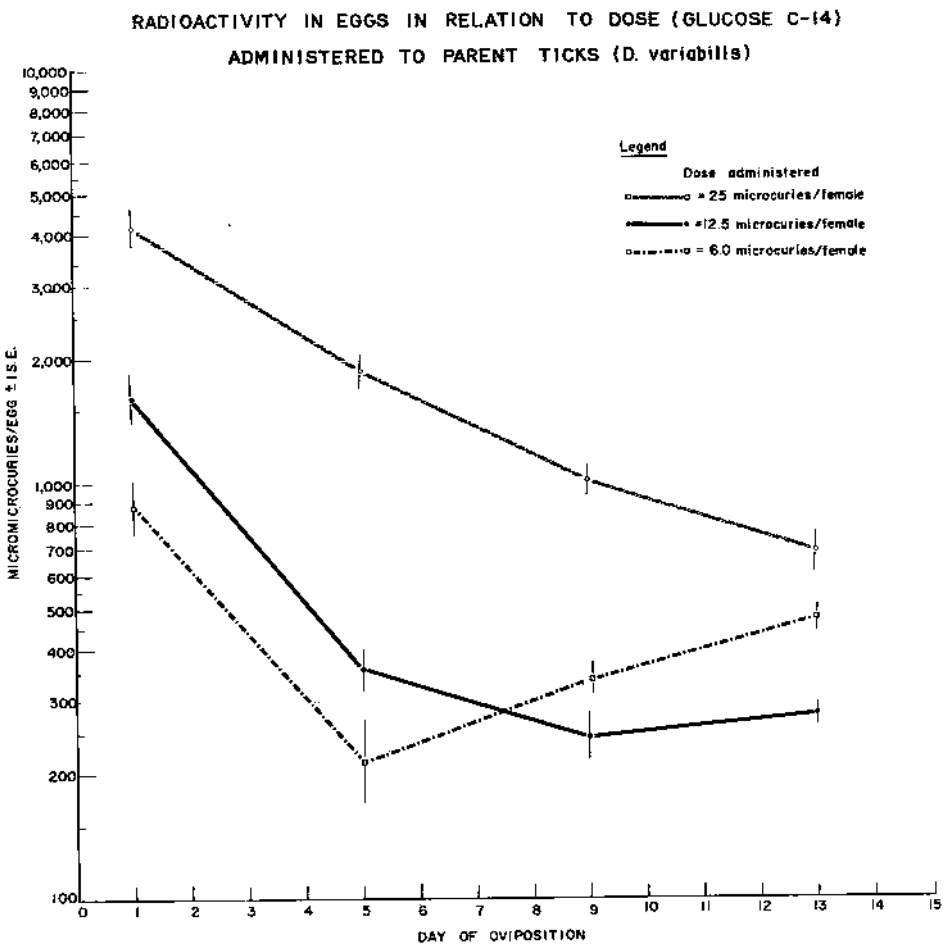

FIG. 3. Graphs showing relative differences in radioactivity in eggs from glucose ^{14}C treated D. variabilis females in relation to dose used. Each point represents average of three samples from oviposits of each of 6 to 7 female ticks inoculated with the same dose. All estimates corrected for difference in efficiency between direct counting (radioactivity) and counting from dissolved material.

Although a large proportion of the eggs died, apparently as a result of the lethal effects of the high radioactivity levels used, many individual larval specimens which hatched had high count rates. Larvae with counts as high as 511 counts/min per larva over background were detected. In one sample, drawn from the progeny of a ^{14}C-glucose treated parent, 54% of the larvae had counts higher than 100 counts/min per larva over background.

Comparisons between radionuclides. The radioactivity observed in eggs and larvae obtained from D. variabilis females treated with seven radiochemical solutions, representing six radionuclides, is shown in Table II. In addition to the high activities obtained in eggs and larvae from parents treated with ^{14}C compounds, considerable activity relative

TABLE I

The relationship between hatching of radioactive egg masses and the amount of radioactivity administered to engorged female Dermacentor variabilis

A. Inoculations with Carbon-14 glucose (uniformly labelled)

Replicate No.	Percent hatching with inoculations of: 12.5 μCi	25.0 μCi
1	49.8	0.0
2	69.1	32.3
3	73.4	33.4
4	74.2	50.1
5	34.8	56.3
6	--	13.8
Mean	60.3	31.0

B. Inoculations with Carbon-14 glycine (uniformly labelled)

Replicate No.	Percent hatching with inoculations of: 10.5 μCi	25.0 μCi	50.0 μCi	60.0 μCi
1	80.0	57.7	64.8	39.4
2	--	58.0	35.6	2.6
3	--	90.9	--	0.0
4	--	82.0	--	0.0
5	--	73.0	--	--
6	--	59.8	--	--
7	--	54.0	--	--
Mean	--	67.9	50.2	10.5

Dash indicates that the proportion hatching was not determined.

to the small size of the dose was detected in the progeny of ticks treated with strontium-90. This was also true of ticks inoculated with phosphorus-32, but those females died very quickly after producing small oviposits, and most eggs failed to hatch. Further studies with ^{90}Sr are in progress.

Efficiency of radiolabelling technique. Knowledge of the efficiency of this method of mass production of tagged ticks was sought to facilitate determination of the dosage for any given radiolabelling problem. To

TABLE II

Radioactivity in progeny (eggs and larvae) of D. variabilis females inoculated with seven different radiochemicals

Radiochemical	No. Inoculations	Mean activity Inoculation (μCi)	Mean radioactivity (all ticks) in CPM over background	
			Mean all eggs	Mean all larvae
Carbon-14 Glycine	33	38.5	91.4	45.0
Carbon-14 Glucose	7	17.5	122.0	93.1
Cerium-144	1	0.04	2.5	*
Cesium-137[a]	3	0.09	8.5	*
Strontium-90[b]	4	0.05	18.5	9.5
Phosphorus-32	6	5.8	211.6[c]	26.0
H^3 Glycine	4	25.0	6.0	*

CPM = Counts per minute

[a] Activity determined with gas flow counter. Activity also determined with solid scintillation detector, and with very similar results.

[b] Including 1 A. americanum

[c] Egg laying ceased shortly after day 1, parent ticks died.

* Not determined.

evaluate this efficiency, it was necessary first (1) to determine the accuracy of the radioactivity values reported by the manufacturers of commercial preparations used in this research, (2) to check the efficiency of the sample preparation technique, and (3) to determine the proportion of the radiochemical dose actually received by the replete female tick prior to oviposition.

Two commercial preparations of the same radiochemical (^{14}C glycine) and from the same supplier were tested. The results of the tests indicated a deviation of 5.6% and 1.8%, respectively, from the reported activity present in each source solution. The efficiency of the sample preparation technique was checked by using 100 lambda aliquots of the previously assayed commercial preparations, added to each of three non-radioactive female tick solutions. Since no inoculations were made

TABLE III

Efficiency of the preparation technique for determining the amount of radioactivity in engorged female Dermacentor variabilis*

Sample No.	Radioactivity in counts per minute over background		
	Tick No. 1	Tick No. 2	Tick No. 3
1	3,990.3	4,365.4	4,379.8
2	2,938.0	4,536.9	4,907.5
3	9,644.7	4,878.3	4,530.3
Mean	4,605.8	4,593.5	4,605.8

Mean all 3 tick solutions - 4,614 CPM over background. True activity = 22,377.9 DPM.

Expected activity (based on an assay value of 50.9 microcuries in original solution) = 6,650.9 $\pm$ 67 counts per minute, or 0.0145 microcuries.

Observed activity (in microcuries) = 0.0101 microcuries.

Efficiency of detection is 69.7%.

* 100 lambda aliquots of the radioactive glycine source solution were withdrawn and pipetted into each fully dissolved, neutralized solution, followed by 2 rinses. Samples were prepared after allowing for equilibrium to be reached.
DPM = Disintegrations per minute

in the case of these three individual ticks, it should have been possible to detect all of the added activity following appropriate dilutions to permit preparation of samples of minimal thickness. In fact, only 69.7% of the predicted activity was detected (Table III). The sample thickness of the preparations studied ranged from 1.3 to 2.5 mg/cm^2. According to Wang and Willis [8], loss of detection efficiency at such sample thicknesses is only about 2-3%. Hence, self-absorption is probably not significantly involved, and the error determined above is accepted as indicative of the true efficiency error in typical samples prepared from all the replete female ticks. The correction factor of 1.44, computed from these data, expresses this deviation between the accuracy of the assay of pure radiochemical versus the mixture of radiochemical and

TABLE IV

Efficiency of technique of direct inoculation of Carbon-14 labelled compounds into engorged female ticks

Radioactivity (Microcuries) reported inoculated	Estimated activity in tick (microcuries)	Percent efficiency (uncorrected)	Percent efficiency (corrected)
10.5	0.182	1.7	2.5
12.5	0.318	2.5	3.6
12.5	1.260	10.1	14.5
12.5	1.818	14.5	20.9
12.5	1.206	9.7	14.0
25.0	3.974	15.9	22.9
25.0	4.030	16.1	23.2
25.0	2.944	11.8	17.1
60.0	10.800	18.0	25.9
Mean 24.2	2.948	11.1	17.7

tick material, and this correction was applied to all the efficiency estimates given below and in Tables IV and V.

The results of the determination of radioactivity present in inoculated engorged female ticks prior to oviposition are shown in Table IV. Nine ticks were studied. The corrected estimates of the efficiency of these inoculations varied from 3.6 to 25.9%, with a mean of 17.7%. In ten other ticks in which the efficiency of transmission to eggs was determined, the cumulative total of activity in both eggs and spent parents indicated an inoculation efficiency of between 15.3% and 75.6%. The overall average efficiency of inoculation, based on all 19 specimens, was estimated at 35.1%.

Data on the relative efficiency of transovarial transmission of the two radiochemicals used by inoculated parents were obtained and are summarized in Table V. Only ticks in which the entire egg mass could be collected and in which a typical size oviposit occurred were used. The entire egg masses were dissolved prior to hatching. The mean efficiency of transovarial transmission of radioglucose and radioglycine in the 10 ticks studied was 60.6%. The mean proportion of the original inoculation

TABLE V

Transovarial transmission of radiochemicals (Carbon-14 labelled glycine and glucose) in Dermacentor variabilis

Radioactivity (microcuries) inoculated	Activity in microcuries*			Efficiency (%)		
	Total[a] activity received (parent)	Total remaining in parent	Total transferred to eggs	Efficiency of inoculation (parent)	Efficiency[b] of transmission to eggs	Efficiency[c] of transovarial transmission
Glycine						
60.0	31.595	27.760	3.835	52.7	6.4	14.0
60.0	17.676	12.548	5.128	29.5	8.5	29.0
25.0	9.006	7.197	1.809	36.0	7.2	20.1
Glucose						
12.5	1.915	0.755	1.160	15.3	9.3	60.6
12.5	5.535	1.071	4.464	42.8	35.7	80.7
12.5	9.258	1.539	7.718	74.1	61.7	83.4
12.5	7.718	2.138	5.580	61.7	44.6	72.3
25.0	18.901	2.344	16.557	75.6	66.2	87.5
25.0	18.602	3.882	14.720	74.4	58.8	79.1
25.0	15.958	3.345	12.613	63.8	50.5	79.0

* All estimates corrected by a factor of 1.44 (see text).

[a] Cumulative total eggs plus spent parent.

[b] Estimate of proportion of original dose which was incorporated in the eggs.

[c] Estimate of the proportion of the dose received by the preovipositing female tick which was transferred to the eggs.

dose which reached the eggs was 34.9%. The total activity present in both spent parent and total egg mass indicates an average efficiency considerably greater than the 17.7% obtained for the nine non-ovipositing females described above. In the later group (ten egg-laying females), the results indicated an inoculation efficiency of 52.6%. It may also be noted that in the three ticks inoculated with radioglycine, the amount of radiochemical remaining in the spent parent was 4.6 times the amount transferred to the eggs. However, in the ticks inoculated with radioglucose, 77.5% of the total activity estimated to have been received by the parent tick was transferred to the progeny.

Feeding. The proportion of radiolabelled D. variabilis larvae which fed as compared with non-labelled larvae feeding on the same host species was studied. The radioactive larvae had count rates averaging

TABLE VI

Comparison of the Feeding of Labelled (Carbon-14) and Non-labelled D. variabilis Larvae on Albino Rats

Radioactive Ticks			Non-labelled Ticks		
No. exposed	No. recovered	% Feeding	No. exposed	No. recovered	% Feeding
50	8		50	15	
50	18		50	2	
50	12		50	11	
50	12		50	9	
50	14		50	23	
50	16		50	7	
50	5		50	12	
Total 350	85	24.3%	350	79	22.6%

110 counts/min per larva. The mean proportion of radioactive larvae which fed was 24.3%; that of non-labelled larvae which fed was 22.6% (Table VI).

Survival. Studies were performed on survival of D. variabilis immatures under controlled conditions of 95% r.h. and 80± 2°F, with both radiolabelled and non-labelled larvae (Table VII). Samples of larvae held under these conditions survived for up to 70 days. The elapsed time when 50% of the non-labelled ticks died was 62 days. The elapsed time when 50% of the radioisotope-tagged larvae died was 63 days. Evidence of long survival of radioisotope-tagged larvae was also obtained from field data. A total of 46 individuals released between 11 August and 29 August, 1966, was recaptured on mice (white-footed mice and a meadow vole) from 14 March through 11 April, 1967, indicating survival followed by host-seeking behaviour for approximately 8 months [7].

Survival of D. variabilis immatures at high temperature (80°F) is limited. However, at lower temperatures, e.g. 50°F, survival of most individuals in an oviposit for up to 8 months has been observed.

Bioelimination. The mean activities detected in larval samples drawn from a stock population at 2-week intervals is shown in Table VIII. Little change in radioactivity appears to have occurred during this period. The survival of D. variabilis larvae at the temperature used is limited, as noted above, and the study could not be continued beyond the sixtieth day. Additional evidence of long-term retention of radioactivity

TABLE VII

Survival of radioactive and non-radioactive *D. variabilis* larvae at 80 ± 2°F and 95% r.h.

Day of Observation	Number of survivors	
	Radioactive	Non-radioactive
1	100	100
14	97	99
35	96	89
42	86	88
45	80	83
50	80	80
52	80	76
54	80	70
55	67	70
56	64	70
60	64	62
62	64	50
63	38	43
65	20	31
69	5	12
70	0	12
72	-	1
75	-	0

was obtained from the field collection noted above. Individual specimens with count rates as high as 59 counts/min were recaptured on the host animals trapped in the release area, indicating retention of activity up to 8 months.

Transtadial transmission. Samples of newly hatched larvae from the second-through-fourth day oviposit of a single *D. variabilis* female were tested for radioactivity. The average number of counts per minute over background per larva was 106.1. These larvae were allowed to feed on albino rats. The average activity in a sample of 50 engorged larvae was 51.4 counts/min per larva over background, with a range of from 7 counts/min per larva to 108 counts/min per larva. A sample of 25 nymphs which moulted from these engorged specimens was counted separately, as were the shed skins. The average radioactivity in the nymphs was 38.4 counts/min per nymph over background. The average activity in the shed skins was 62.7 counts/min per larval skin over background (Table IX).

4. DISCUSSION

The inoculations with radioglucose reported in this paper resulted in oviposition by 93.3% of the treated ticks. Hence, oviposition by

TABLE VIII

Retention of radioactivity by fasting
Dermacentor variabilis larvae
stored for long periods

Day	Sample No.[a]	Avg. CPM/larva over background
0	1	46.7
14	2	38.8
28	3	42.3
42	4	58.8
56	5	27.5
70	6	49.3

[a] Each sample contained 10 specimens. All were living specimens, except the sample taken on the 70th day. All remaining larvae died between the 56th and 70th day; these specimens were segregated into three groups of 10 each, counted, and the mean activity was computed for the three samples.

almost all of the ticks radiolabelled by this method may be expected when the osmotic pressure of the carrier solution is adjusted to a value more closely resembling that of the haemolymph. The ability to lay eggs does not appear to be curtailed by the in-vivo incorporation of soft beta-emitting radiation.

This method of radiolabelling immature ticks appears to offer more advantages than other techniques reported in that (1) the radioactive material is incorporated into the bodies of the tagged specimens and, hence, it is not readily lost, (2) it is simple to apply, (3) it is not wasteful of material, and (4) the size of the dose administered can be controlled.

Radiosensitivity of the eggs represents one of the major limiting factors on the usefulness of the radioinoculation method for tagging immature ticks. No hatching occurred in oviposits in which the average count rate per egg exceeded 637. Hatching occurred at lower activity levels, but was quite obviously related to the radiochemical and the dose administered (Table I). Grosch [9, 10] also found reduced egg laying and diminished hatching in the wasp (Habrobracon sp.) fed on radiophosphorus and zinc-65. Another important limitation on the use of this method for mass rearing of tagged ticks is the progressive decline in radioactivity with the period of oviposition by the parent ticks (Fig. 1). The reason for this is unknown. Consequently, progeny produced late in the egg-laying period were often found to have radioactivity levels which were unsatisfactory for field studies.

TABLE IX

Radioactivity (Carbon 14) in engorged larvae, nymphs, and shed skins of D. variabilis[a]

	Radioactivity in counts per minute over background		
Tick No.	Engorged Larvae	Nymphs molted from Engorged Larvae	Larval Skins
1	22	39	308
2	12	49	65
3	37	33	113
4	36	47	20
5	70	29	39
6	106	10	33
7	22	20	37
8	21	6	19
9	29	32	29
10	64	28	37
11	23	3	13
12	107	34	92
13	86	40	72
14	59	46	43
15	73	32	45
16	32	11	43
17	84	--*	--*
18	60	23	89
19	22	92	83
20	75	56	116
21	36	13	29
22	106	87	98
23	96	35	65
24	67	38	78
25	63	33	39
Mean ± 1 S. E.	56.7 ± 6.1	34.8 ± 4.4	62.7 ± 6.3

[a] Larval progeny of female No. 17, inoculated with approximately 50 μCi Carbon-14 glycine. Larvae were collected from the oviposits of days 2 through 4. The average number of counts per minute over background per unfed larva was 106.1.

* Died without moulting.

The enormous reproductive capacity of ixodid ticks compensates for the losses noted above. The estimated average number of eggs produced by 15 untreated D. variabilis females was 4156. The maximum number observed was 6173. Radioinoculated females produced only slightly fewer eggs. The average number for 48 ticks was 4002. Another important consideration is the temporal distribution of the egg laying process. Figure 4 shows the average daily egg production of 15 D. variabilis females held in the dark at 85% r.h. and 80±2°F. By referring to Fig. 4, it may be found that 59.2% of the eggs are deposited by the end of the fifth day of oviposition, and all but 6% are laid by the end of the

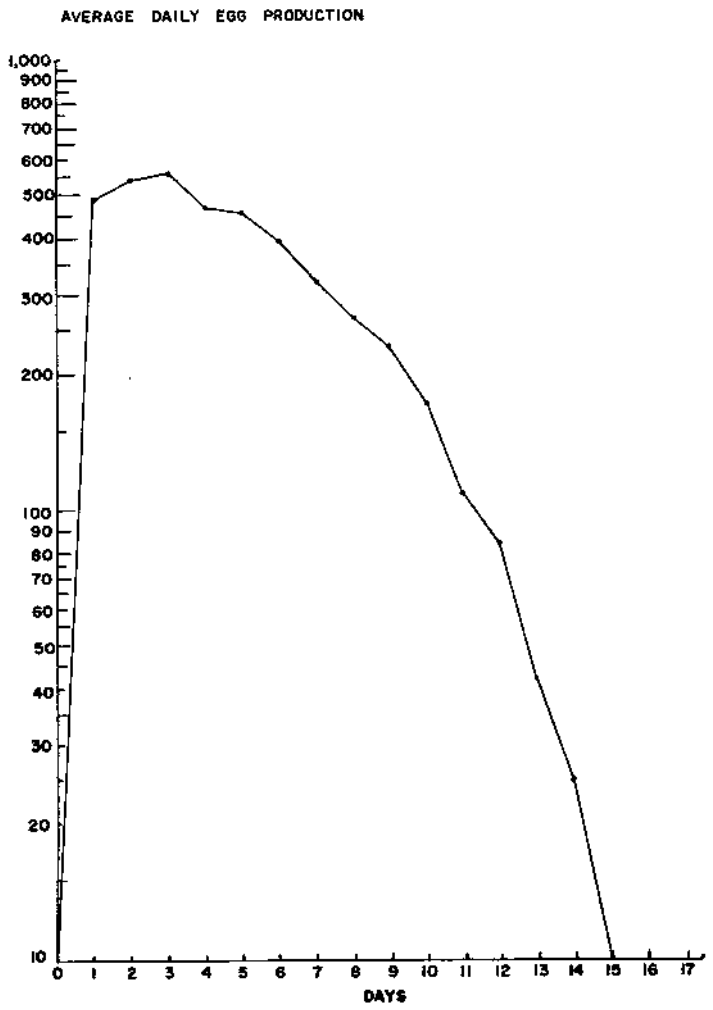

FIG. 4. Average daily egg production of 15 D. variabilis females held at 80 ± 2° F. and 85% relative humidity.

tenth day. This knowledge of the characteristics of the egg-laying process in ticks may be used, along with information on the efficiency of inoculation, transovarial transmission, and loss of activity with hatching, to determine the best method of mass rearing of ticks for use in field studies.

Preparation of the release population. An example of the application of the method for mass rearing of radioisotope-tagged tick populations may be given by assuming the objective of producing 100 000 D. variabilis larvae with an average radioactivity of not less than 50 counts/min per larva. The radiochemical selected is glucose ^{14}C. Since approximately half of the radioactivity incorporated in the eggs is lost during hatching, the inoculating dose per parent tick must be adjusted to compensate for it. By referring

TABLE X

Determination of the dose (in microcuries) of Carbon-14 glucose necessary to radioactivate the maximum number of D. variabilis larvae with a minimum of 100 CPM/larva over background

Day of oviposition	Avg. No.[a] Eggs	Avg. expected[b] activity (CPM)	Activity in total oviposit (microcuries)		Activity/ larvae (CPM)
1	483	260	0.274		128
2	537	220	0.258		108
3	552	180	0.218		88
4	467	140	0.143		69
5	452	100	0.099		49
6	392	90	0.077	DISCARDED	
7	320	79	0.055		
8	266	68	0.039		
9	230	57	0.029		
10	172	53	0.020		
11	107	50	0.012		
12	84	46	0.008		
13	42	43	0.004		

[a] Data from Figure 4.

[b] Data from Figure 1.

to Fig. 1, it may be observed that the progressive decline in radioactivity per egg with time of oviposition is linear, with a midpoint near the fifth day. Hence, the activity of the fifth day oviposit may be used as the base level for determining the dose. Eggs produced after this day are discarded. The dose required to activate the fifth-day oviposit to a level of 100 counts/min per egg over background is 0.099 microcuries (average of 452 eggs × 100 counts/min × detector efficiency, or 100/20.6, divided by the number of disintegrations per minute per microcurie, or 2 220 000). The activities required for the other oviposition periods can also be determined from the data in Figs 1 and 4. These calculations are shown in Table X. When the activity of the radiochemical which may be expected to remain in the spent parent is included (Table V), the total activity which must be received by the preovipositing female is 1.594 μCi. Since the efficiency of inoculation has been estimated to be 35.1%, the dose required to obtain the desired minimal level of labelling of the maximum number of larvae is 4.54 μCi. The final expected yield of larvae, taking into account the loss due to radiosensitivity (Table I), is 1 868. Inoculation of a total of 57 engorged female ticks will be necessary to obtain the required number of larvae.

In practice, the investigator may wish to increase the dose to ensure adequate labelling. Further, a monitoring check of the hatch from each

oviposit which is scheduled for use as a component of the release population should be included in the laboratory protocol to ensure that all released ticks are suitably labelled.

Biological characteristics of radioisotope-tagged ticks. An important consideration which bears upon the application of radiolabelling methods in ecological studies is whether the biological characteristics of radioisotope-tagged individuals are similar to those of untreated specimens. The data reported in this paper suggest that they are very similar. No apparent detrimental influence was noted when feeding of radioactive larvae was compared with that of non-radioactive larvae. The proportion of the ticks (exposed to a host, albino rat) which fed was almost identical in each case, and it was not significantly different from the proportion reported by Sonenshine and Atwood [11] for this same host. Survival of radioisotope-tagged larvae appears to be similar to survival of non-labelled larvae. The elapsed time when 50% mortality occurred was almost identical in both cases (62 and 63 days, respectively, at 80°F and 95% r.h.). In addition to laboratory evidence, recaptures of overwinter survivors after a period of up to 8 months were made. Larvae released in August 1966 at a study site near Montpelier, Virginia, were recaptured on small mammals in March and April 1967 [7]. Thus, all the evidence obtained to date suggests that radiolabelled ticks may be regarded as similar to non-labelled laboratory reared ticks. However, this does not imply that they are similar to wild individuals living in the natural environment. Further study with laboratory reared populations released in natural and simulated environments will be needed to determine the degree of behavioural similarity between these ticks and the native ones, as well as to determine the proportion of the released population which survives from one year to the next.

In addition to determining whether radioactive ticks were biologically similar to non-labelled individuals, it is also of interest to determine how long the radioactive label remains in each stage. In free-living insects, bioelimination often accounts for more loss of radioisotopic material than nuclear disintegrations. The biological half-life of a radionuclide is very different from the physical half-life [12]. In ticks, however, there is no daily exchange of nutrient and waste material during the non-parasitic periods. Consequently, long-term retention of radioactivity may be expected. The data in Table VIII lends support to this hypothesis. Unfortunately, further study of retention of radioactivity by fasting larvae could not be continued at the temperature used (80°F) because of death of all of the specimens. Further investigation of this problem will be done with ticks held at lower temperatures. Data from the field study also support the expectation of long-term retention of radioactivity. Overwinter survivors were collected which had activities as high as 59 counts/min per larva over background.

Radiolabelling of nymphs results from moulting of fed labelled larvae (Table IX). The activity levels found in these nymphal ticks were adequate for detection of most individuals. The data suggests that populations of tagged nymphs suitable for use in field studies may also be produced by this method.

5. SUMMARY AND CONCLUSIONS

The feasibility of mass rearing radioisotope-tagged immature ticks by means of inoculating engorged females with radiochemicals is demonstrated in three species of Ixodidae. The presence of radioactivity may be detected readily in individual larvae or nymphs.

The incorporation of radiochemicals internally into ticks reduces the chance of losing the radioactive label when moulting occurs, or under natural conditions. Further, the amount of radioactivity in the specimens can be controlled by controlling the size of the dose administered to the parent ticks. However, differences in radioactivity in the progeny of treated females in relation to the day of oviposition were noted. This activity declined progressively through the ninth day. Differences were also noted in relation to the radiochemical used. Most of the ^{14}C glycine (79.0%) received by the engorged females remained in the parents, whereas most of the ^{14}C glucose (77.5%) received was transferred to the progeny. Hatching of eggs labelled by this method was less than in untreated oviposits. The degree of radiosensitivity was related to the amount of radioactivity in the dose given to the parents. Large doses were lethal to many eggs. No hatching occurred when the average activity of the labelled eggs was 637 counts/min per egg over background, or greater. Nevertheless, numerous highly radioactive larvae hatched, including individuals with counts as high as 511 counts/min per larva over background.

The biological characteristics of the tagged larvae which hatched did not appear to have been affected by the presence of radioactivity. The proportion of marked larvae which attached to hosts was similar to the proportion of unmarked larvae. The time of survival of fasting, radioisotope-tagged larvae, under laboratory conditions, was similar to the period of survival of non-radioactive larvae. No apparent loss in radioactivity was detected in fasting larvae held for up to 70 days under laboratory conditions.

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The author wishes to express his sincerest appreciation to Mr. J. Stout for his assistance in trapping the wild mammal hosts while he was associated with Old Dominion College, Norfolk, Virginia, and to Mrs. J. Tigner, also of this institution, for her assistance in the laboratory studies described in this communication.

REFERENCES

[1] KNAPP, S. E., FARINACCI, C. J., HERBERT, C. M., SAENGER, E. L., A method of labelling the lone star tick (Amblyomma americanum) with a radioactive indicator (P^{32}). J. Econ. Entomol. 49 (1956) 393.

[2] QUAN, S. F., HARTWELL, W. V., SCOTT, K. S., PENG, C. T., Cerium 144 as a tag for arthropods of medical importance. Trans. Royal Soc. Trop. Med. Hyg. 51 1 (1956) 87.

[3] HYLAND, K. E., Jr., HAMMAR, J. L., Transovarial passage of radioactivity in ticks labelled with C^{14} glycine. J. Parasitol. 45 Suppl. 2 (1959) 24.
[4] BABENKO, L. V., The use of radioisotopes for labelling ticks. Med. Parazitol. i Parazitan. Bolezni. 29 (1960) 324.
[5] SAMOSTREL'SKII, A. Iu., DAITER, A. B., Experiment of the use of radioactive isotopes to mark Ornithodoros papillipes ticks. Tr. Inst. Epidem. Paster. 29 (1966) 86.
[6] SONENSHINE, D. E., YUNKER, C. E., Radiolabelling of ticks by inoculation. In preparation.
[7] SONENSHINE, D. E., CLARK, G. M., Field trials on radioisotope tagging of ticks. In preparation.
[8] WANG, C. H., WILLIS, D. L., Radiotracer Methodology in Biological Science (PRENTICE HALL, INC.), Englewood Cliffs, New Jersey (1965) 382.
[9] GROSCH, D. S., SULLIVAN, R. L., The effect of ingested radiophosphorus on egg production and embryo survival in the wasp Habrobracon. Biol. Bull. 102 (1952) 128.
[10] GROSCH, D. S., Distribution of Zinc-65 in the wasp, Habrobracon, and its effect on reproduction. Nature. 195 (1962) 356.
[11] SONENSHINE, D. E., ATWOOD, E. L., LAMB, J. T., Jr., The ecology of ticks transmitting Rocky Mountain spotted fever in a study area in Virginia. Ann. Entomol. Soc. Amer. 59 6 (1966) 1234.
[12] CROSSLEY, D. A., Jr., Consumption of vegetation by insects. Proc. First Natl. Symposium Radioecology. (1963) 427.

DISCUSSION

H. MARCHART (Chairman): Have you estimated the approximate radiation dose received by the ovaries of the female ticks?

D. E. SONENSHINE: No, we do not have the equipment for this. However, I am concerned with radioisotopes inoculated into the females and so far, with doses of up to 60 μCi of ^{14}C per tick, I have not seen any evidence of radiation damage to the ovaries. The average number of eggs deposited by inoculated females was almost the same as that produced by non-inoculated females.

D. S. GROSCH: I feel it should be borne in mind that the energy of the beta rays and their range in tissue are important in investigating the dose level tolerated. For instance ^{14}C, in any form, can be tolerated in much larger quantities than ^{32}P.

I should be interested to hear whether you have any life-span data on the ovipositing females. The pattern on you slides resembled the senile decline seen with Habrobracon and Drosophila — though, of course, the time-scale would not be identical.

D. E. SONENSHINE: The female Ixodid tick lays all her eggs in one brief oviposition period, and dies on, or shortly after, completion of this process, so there is no question of senility here.

D. S. GROSCH: Then could you tell us whether the mean day of death is day 15? I am trying to relate this to something fundamental in insect physiology and I believe that the fat body is very much involved in insect oviposition.

D. E. SONENSHINE: The mean day of termination of egg laying was not computed exactly, but it is certainly around 15 days. The actual

life-span of the female is less significant, since she may well survive for a short period after ovipositing.

Rachel GALUN: I would just like to point out that ticks do not in fact have a fat body.

D. S. GROSCH: Then by what mechanism do those processes occur in ticks which are performed by the fat body in insects?

Rachel GALUN: Ticks utilize blood very slowly and use the gut as a storing place for food.

D. S. GROSCH: However, the insect fat body is more than a place for storing sudan positive material.

D. E. SONENSHINE: This is an interesting point.

W. F. BALDWIN: You mentioned that egg batches laid by females treated with ^{14}C in glucose showed a substantial and progressive decline in radioactivity through the first nine days of oviposition. This drop is of the order of 20% and might well interfere with the study. Could you give us an indication of the reasons for this diminishing degree of radioactivity?

D. E. SONENSHINE: I cannot give a definitive answer to this. However, I could speculate that it may be related to the position of the eggs in the reproductive tract. Perhaps eggs in the oviducts and those in the ovary have different rates of uptake of metabolites.

ETUDE ECOLOGIQUE DE DEUX PUCERONS VECTEURS DES VIROSES DE LA BETTERAVE

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Abstract — Résumé

ECOLOGICAL STUDY OF TWO APHID VECTORS OF THE SUGAR-BEET VIRUS. The dispersal of Myzus persicae Sulz and Aphis fabae Scop. labelled with ^{32}P was studied from 1963 to 1965 in sugar-beet plots.

The aphids were reared on beets infected with a virus causing severe yellowing and sprinkled with a solution containing 1 mCi of sodium orthophosphate (^{32}P). Aphids which remained for 24 hours on these plants were significantly labelled, as were also their first-generation descendants. The activity of the insects was recorded by a gas-flow detector with a low background. The radioactive beets infected with the virus and laden with aphids were placed in the centre of the plots. After successively increasing dispersal times, the largest possible number of aphids was captured in each test, either manually or in traps. The co-ordinates and the precise identification of each labelled aphid were entered on charts, some of which are discussed by the authors.

The experiments demonstrated the mobility of the apterae, which were found at average distances of 6.5 and 7 metres for Myzus persicae and Aphis fabae, respectively. However, some apterae of both species were found at distances of over 15 metres after the same period.

The role of the winged species as virus carries over short distances should not be underestimated.

The active character of both apterae and winged species is emphasized.

ETUDE ECOLOGIQUE DE DEUX PUCERONS VECTEURS DES VIROSES DE LA BETTERAVE. La dispersion de Myzus persicae Sulz et d'Aphis fabae Scop. marqués à l'aide de phosphore-32 a été suivie, au cours des années 1963 à 1965, dans des parcelles de betteraves sucrières.

Les pucerons sont élevés sur des betteraves virosées (infectées par le virus de la jaunisse grave de la betterave) et arrosées d'une solution contenant 1 mCi d'orthophosphate de sodium (^{32}P). Des pucerons ayant séjourné 24 heures sur ces plantes sont significativement marqués; leur descendance de la première génération l'est aussi. L'activité des insectes est décelée par un détecteur à courant de gaz, de grande sensibilité et à faible bruit de fond. Les betteraves radioactives virosées et chargées de pucerons sont placées au centre des parcelles. Après des temps de dispersion croissants, on prélève dans chaque parcelle le maximum de pucerons, soit manuellement, soit à l'aide de pièges. Les coordonnées et l'identité précise de chaque puceron marqué sont reportées sur des plans. Certains de ceux-ci sont commentés par les auteurs.

Les essais ont prouvé la mobilité des formes aptères des deux espèces. Les Myzus et Aphis aptères sont retrouvés, en moyenne, respectivement à 6,5 mètres et à 7,0 mètres de leur point de départ, après 24 heures de dispersion. Certains individus des deux espèces sont capturés à plus de 15 mètres au bout du même laps de temps.

La part d'intervention des formes ailées dans la dissémination des viroses à courte distance ne semble pas négligeable.

Le caractère actif des déplacements des formes aptères et ailées a pu être souligné.

INTRODUCTION

Vecteurs principaux de la jaunisse grave de la betterave (VJGB), Myzus persicae Sulz et Aphis fabae Scop. sont très communs en Belgique.

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Plusieurs chercheurs ont démontré l'efficacité supérieure du premier vecteur: Watson et al. [1] au Royaume-Uni, en comparant l'incidence annuelle de la maladie à l'abondance relative des deux espèces, Bjorling [2] en tirant parti du fait que la virose ne se présente pas en Suède centrale et en comparant directement sa dispersion par chacun des deux vecteurs. Bjorling et Ossiannilsson [3] ont également pu montrer au laboratoire que, malgré des différences dans l'efficacité de transmission des deux espèces, Myzus persicæ détermine de trois à quatre fois plus d'infection qu'Aphis fabæ. Les travaux de Heathcote et Cockbain [4] confirment ces résultats. De plus, ils montrent que les formes ailée et aptère des deux vecteurs transmettent aussi aisément le VJGB.

Les rôles respectifs des formes ailée et aptère dans la dispersion des virus persistants et semi-persistants sont difficiles à déterminer. Davies [5] insiste sur l'importance des aptères dans ce domaine. Broadbent [6], Broadbent et Gregory [7], Broadbent [8, 9] ne partagent pas cette opinion. De nombreux auteurs, cependant, maintiennent qu'une bonne part de la dispersion des virus persistants et semi-persistants au sein du champ est due aux aptères. Ribbands [10] par exemple, pour suivre la dispersion des jaunisses grave et modérée, n'étudie que le comportement des aptères de Myzus persicæ.

Une technique permettant de repérer les aphides s'avérait nécessaire afin de suivre leurs déplacements en champ et de préciser le rôle joué par les formes aptère et ailée. Bjorling et al.[11] utilisèrent le radiophosphore ^{32}P pour marquer les aphides et les repérèrent par autoradiographie. Cependant ils ne réussirent à marquer d'une façon décelable qu'une fraction (évaluée à moins d'un tiers) des individus. Ils obtinrent néanmoins des renseignements très intéressants sur la dispersion des deux vecteurs. C'est en nous fondant sur les travaux de ces chercheurs, mais en utilisant une méthode de détection plus efficace, que nous avons poursuivi ces études au cours des années 1963 à 1965.

METHODES

Les aphides des deux espèces sont élevés ensemble sur des betteraves de la variété Kleinwanzleben E. cultivées en pots de 30 cm de diamètre. Les plantes-sources sont, suivant les années, inoculées ou non de VJGB (souche du Laboratoire de phytovirologie de Gembloux). Quelques jours avant le début de l'expérience, les parcelles sont traitées au moyen d'un insecticide à courte rémanence, tandis que les pots sont mis dans des seaux en polyéthylène et arrosés au moyen d'une solution d'orthophosphate de sodium radioactif (1 mCi par pot et par plante). Le surlendemain, ils sont recouverts d'une coiffe de gaze empêchant toute fuite d'insectes.

Bjorling et al. [11] ont montré que les aphides sont rapidement marqués de cette façon, qu'ils le restent pendant au moins 2 à 3 semaines et qu'une partie de la radioactivité passe à la descendance. Depuis, d'autres travaux sur l'assimilation et l'excrétion du ^{32}P chez les aphides [12-16] ont fait progresser nos connaissances.

Les betteraves ainsi coiffées sont transportées avec précaution deux à trois jours plus tard au milieu des parcelles expérimentales et enfouies à raison de quatre plantes par parcelle. Les coiffes sont alors enlevées et introduites sur place dans de grands sacs en polyéthylène, afin

d'empêcher la dissémination accidentelle des insectes marqués. Les parcelles ont été installées en 1963, 1964 et 1965 dans un champ dégagé, homogène, situé à 100 mètres de la station climatologique de Gembloux. Le nombre et la disposition des parcelles ont varié au cours des trois années. Cependant les conditions du semis n'ont pas été modifiées: 50 cm entre les lignes et plantes distantes de 33 cm dans les lignes. Les lignes de betteraves sont numérotées et une betterave sur 10, dans la ligne, reçoit un numéro d'ordre. Les récoltes commencent généralement le lendemain du transport des plantes-sources au champ. Elles sont faites manuellement. Une équipe de capture comprend un récolteur et un aide. Le premier découvre, identifie et prélève le puceron au moyen d'un pinceau. Le second porte la planchette où sont collées les cupules destinées à le recevoir et note les coordonnées de la plante et le numéro d'ordre de la cupule. Les cupules sont adaptées à l'équipement de comptage et ont reçu, au préalable, une goutte de glycérine et d'insecticide. Elles sont disposées dans le passeur d'échantillons sans autres préparatifs. De nombreux pièges jaunes sont également disposés dans les parcelles et les aphides ailés qui y sont trouvés sont placés dans les mêmes cupules en vue du comptage.

La radioactivité des insectes est établie au moyen d'un détecteur Geiger-Müller à courant de gaz, à faible mouvement propre et de grande sensibilité (fenêtre très mince de quelque 0,125 mg par cm^2) couplé à une échelle de comptage adéquate[1]. L'emploi d'un passeur automatique (capacité: 50 unités) et d'une calculatrice imprimante nous a permis de mesurer l'activité de milliers d'insectes en quelques semaines et d'obtenir ainsi rapidement les informations nécessaires tout en menant les récoltes sur une grande échelle. Suivant ce processus, il a été possible de traiter près de 4 000 pucerons en 1963, quelque 10 000 en 1964 et 11 000 en 1965. Les échantillons sont comptés pendant trois minutes. Seuls les pucerons donnant, après trois comptages, une mesure reproductible supérieure à trois mouvements propres sont considérés comme radioactifs. A chaque début d'essai, on prélève un échantillon des aphides se trouvant sur les plantes radioactives. Dans tous les cas, les insectes soumis au comptage sont significativement marqués. Les résultats sont alors reportés sur les plans des parcelles.

ESSAIS DE 1963

Ces essais, destinés principalement à la mise au point des modalités expérimentales, ont néanmoins donné des indications intéressantes sur la dispersion des deux vecteurs.

Quatre parcelles de betteraves sucrières (δ, γ, α, β,) de 20 m × 20 m ont été établies dans un champ d'avoine, la distance entre les parcelles étant de 15 m. Les plantes-sources, chargées des deux espèces aphidiennes et inoculées de VJGB, ont été introduites dans les parcelles respectivement les 9, 16, 29 juillet et 7 août 1963.

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[1] Tracerlab « Omni-guard» low-background counting system. Mouvement propre: 1 cpm; diamètre du détecteur: 25 mm; rendement du détecteur voisin de 25% dans les conditions de nos comptages.

Parmi les 1084 Myzus persicæ capturés dans les quatre parcelles, 14, soit 1, 3%, étaient marqués (6 ailés et 8 aptères). En ce qui concerne Aphis fabæ, 135 individus marqués (25 ailés, 86 aptères et 24 formes «immatures») ont été dénombrés dans un échantillonnage de 2719 individus, ce qui représente près de 5% des captures.

Les données obtenues sur la dispersion des insectes sont encore assez limitées. Les aptères ont été, pour la plupart, retrouvés à proximité immédiate des plantes-sources, dans un rayon ne dépassant pas 1 m, au cours des trois premiers jours suivant le lâcher. Notons cependant qu'un Myzus persicæ aptère a été découvert à 12 m des plantes-sources, 2 jours après le lâcher, et qu'un Aphis fabæ aptère a été capturé à une distance de 2, 3 m après 1 jour de dispersion.

La figure 1 donne, à titre indicatif, la dispersion de la jaunisse observée 4 semaines après le lâcher des aphides dans une des parcelles de l'essai (parcelle α, lâcher du 16 juillet 1963).

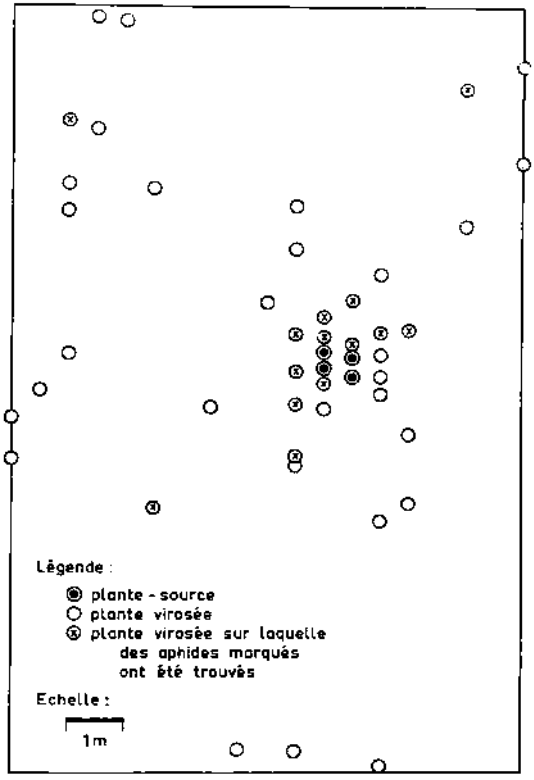

FIG. 1. Essais de 1963, parcelle α : distribution des plantes présentant les symptômes du VJGB et localisation des aphides marqués capturés.

Comme on pouvait s'y attendre [10], la densité des plantes virosées est plus élevée à proximité des plantes-sources. De plus, les plantes sur lesquelles un aphide radioactif a été capturé ont développé les symptômes de la maladie.

ESSAIS DE 1964

En 1963 les captures, étalées dans le temps, avaient été effectuées sur la même parcelle et avaient pu influencer la dispersion ultérieure des aphides. En outre cette façon d'opérer augmentait les risques d'entraînement accidentel des aphides marqués.

Les essais de 1964 ont été conçus pour vérifier les observations faites l'année précédente sur la mobilité des deux espèces aphidiennes considérées, tout en évitant les inconvénients en question.

Cinq parcelles rectangulaires identiques (40 m ×10 m) dont le grand axe était orienté suivant la direction des vents dominants, ont été enclavées dans un champ d'avoine. Quatre betteraves-sources ont été placées au centre de chaque parcelle, au début de chaque essai, et nous avons admis par hypothèse que la dissémination des aphides se fait de la même façon dans toutes les parcelles. Les prélèvements d'insectes ont eu lieu, dans une première parcelle, 24 h après le lâcher des pucerons. Les lignes centrales ont été prospectées betterave par betterave, les autres par coups de sonde en fonction des disponibilités en personnel. Le soir même, cette première parcelle a été traitée avec une solution à 0,3% de Phosdrin. Les autres parcelles ont été prospectées et traitées de la même façon respectivement 2, 3, 4 et 6 jours après le lâcher. Des pièges jaunes ont été disposés le long du côté nord-est de chaque parcelle et ont été dépouillés chaque jour. Quatre essais se sont succédé sur ces cinq parcelles. Ils ont débuté respectivement les 1er, 15, 29 juillet et 5 août 1964. Pour ce dernier essai, les captures n'ont eu lieu que 1 jour après le lâcher. Les conditions climatiques ont été, dans l'ensemble, favorables à la pullulation des pucerons.

La figure 2 donne la localisation des ailés, des aptères et des formes immatures des deux espèces capturées manuellement et dans les pièges jaunes après 1 jour de dispersion (2^{e} essai) et l'effectif détaillé de l'échantillon de sous-parcelles idéales de 2,5 m de côté.

La figure 3 rassemble toutes les captures effectuées après 1, 2, 3, 4 et 6 jours de dispersion au cours de ce deuxième essai (15 - 21 juillet). Enfin, dans les tableaux I et II sont cumulées les captures d'individus aptères et ailés, réalisées au cours des quatre essais après 1 et 2 jours de dispersion, les aphides étant classés en fonction de leur éloignement par rapport à leur point de départ.

Aptères

Les aptères, tant ceux d'Aphis fabæ que ceux de Myzus persicæ, sont très mobiles: neuf Aphis fabæ sur 17 ont été retrouvés à plus de 5 m des plantes-sources après 2 jours de dispersion, alors que ce rapport a été de 33 individus sur 100 chez Myzus persicæ.

Trois Aphis et six Myzus ont été retrouvés à plus de 15 m de leur point de départ après 2 jours de dispersion.

Ailés

Les ailés des deux espèces ont été retrouvés dans les parcelles à des distances variables et, en ce qui concerne les captures manuelles, dans

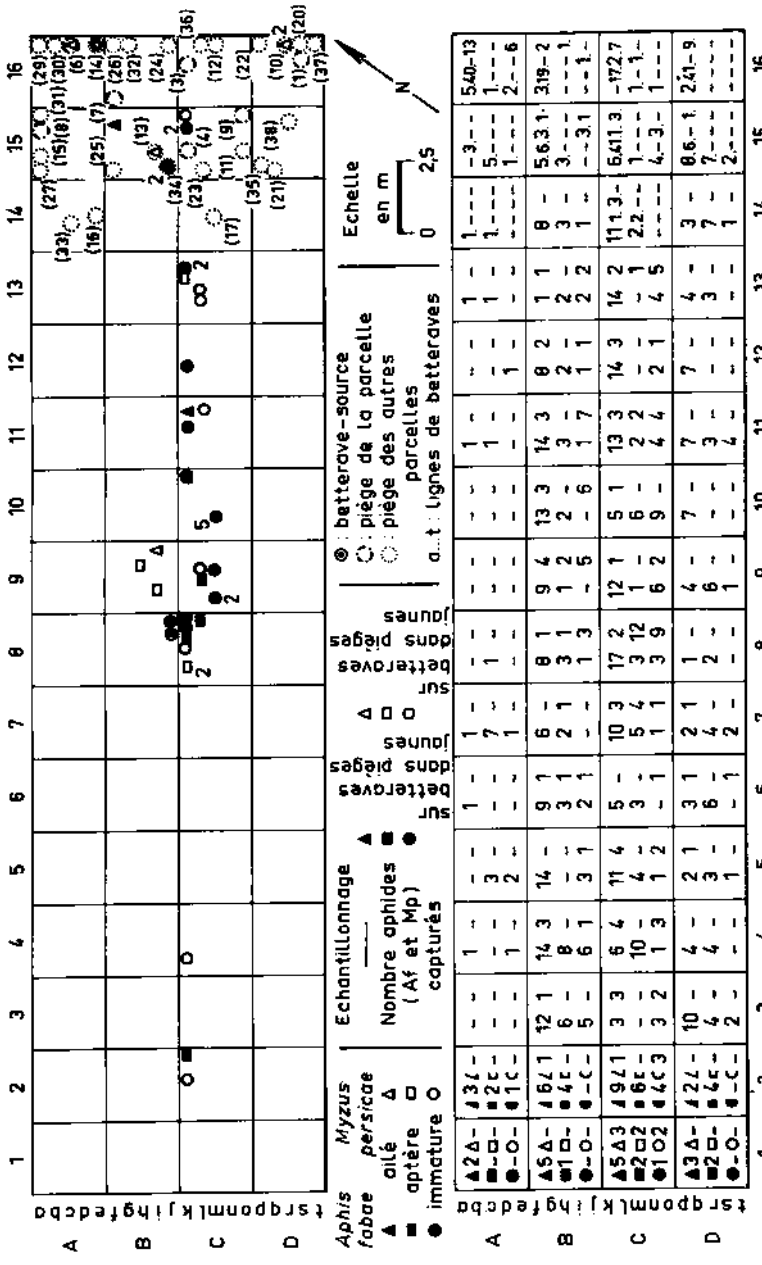

FIG. 2. Essais de 1964: captures après 1 jour de dispersion (deuxième essai, 16 juillet).

FIG. 3. Essais de 1964: captures après 1, 2, 3, 4 et 6 jours de dispersion (deuxième essai, 16-21 juillet).
Aphis fabae: ▲ ailé, ■ aptère, ● immature
Myzus persicae: Δ ailé, □ aptère, ○ immature
×: betterave-source
⊙: piège jeune
I-VI: jours après le lâcher.

TABLEAU I. REPARTITION DES APTERES CAPTURES APRES 1 ET 2 JOURS DE DISPERSION EN FONCTION DE L'ELOIGNEMENT PAR RAPPORT AUX PLANTES-SOURCES
Essais de 1964: captures cumulées des quatre essais

Distance (m)	Myzus persicæ		Aphis fabæ	
	1 jour	2 jours	1 jour	2 jours
0 - 5	21	46	4	4
5 - 10	8	8	-	2
10 - 15	8	3	2	2
15 - 20	4	2	1	2
	41	59	7	10
Aptères radioactifs	100		17	
Total des captures	349		468	

TABLEAU II. REPARTITION DES AILES (CAPTURES MANUELLES ET PIEGES) CAPTURES APRES 1 ET 2 JOURS DE DISPERSION EN FONCTION DE L'ELOIGNEMENT PAR RAPPORT AUX PLANTES-SOURCES.
Essais de 1964: captures cumulées des quatre essais

Distance (m)	Myzus persicæ		Aphis fabæ	
	1 jour	2 jours	1 jour	2 jours
0 - 5	4	2	1	7
5 - 10	-	1	1	-
10 - 15	-	-	-	2
15 - 20	2	-	1	-
Pièges à 20 m	5	-	3	5
	11	3	6	14
Ailés radioactifs	14		20	
Total des captures	544		1476	

des directions opposées. Elles représentent respectivement 14% et 118% des capture d'aptères chez Myzus persicæ et chez Aphis fabæ.

Formes immatures

Ces formes ont été retrouvées dans les parcelles dans toutes les directions et à des distances variables des plantes-sources. Il n'est pas

possible de faire la distinction entre celles engendrées par les aptères sur les plantes radioactives ou après dispersion et celles descendant des alatæ virginipares. De toute façon, les distances auxquelles ces formes immatures ont été retrouvées après 24 h de dispersion sont du même ordre de grandeur que celles observées pour les formes ailées et aptères.

ESSAIS DE 1965

L'exiguïté des parcelles utilisées en 1963-64 impliquait certains inconvénients: le microclimat ainsi réalisé peut fausser partiellement la dispersion et modifier les conditions de vol des pucerons. Pour y remédier, nous avons établi en 1965 un champ de betteraves d'une superficie de 1 ha 40 a au centre duquel une parcelle carrée de 60 m de côté était piquetée (soit environ 130 lignes de betteraves). Des pièges jaunes (144 au total) ont été disposés selon quatre cercles concentriques à 20, 28, 40 et 60 m des betteraves radioactives. Celles-ci, chargées de colonies des deux espèces, ont été amenées au centre de la parcelle le 20 juillet et le lâcher des pucerons a eu lieu à 11 h. Le relevé des pièges jaunes a été effectué le jour même entre 14 et 17 h ainsi que les 22, 23, 26, 27 et 28 juillet entre les mêmes heures. Les captures manuelles ont débuté le 21 juillet, les prélèvements se faisant sur les 10e, 20e, 30e ... betteraves des lignes paires de la moitié supérieure de la parcelle (voir fig. 4). Le 22 juillet l'autre moitié de la parcelle a été prospectée de la même façon. Le 3e jour (23 juillet), les captures ont été faites sur les 11e, 21e, 31e... plantes des lignes centrales (lignes 49 à 80). De fortes pluies ont interrompu les captures, qui ont été reprises le 26 juillet, soit après 6 jours de dispersion, sur les 7e, 17e, 27e... plantes des lignes impaires de la partie supérieure; elles ont été poursuivies le 27 juillet, selon le même schéma, dans la partie inférieure de la parcelle. Enfin, le 8e jour (28 juillet), les captures ont été effectuées sur toutes les plantes d'un carré central de 10 m de côté.

Les conditions climatiques de l'été 1965 ont été particulièrement inclémentes. Les observations météorologiques faites à Gembloux au cours de la troisième décade de juillet sont groupées dans le tableau III.

Les figures 5 et 6 reproduisent les anémogrammes (vitesse et direction des vents) concernant la période envisagée (20-28 juillet 1965). La figure 5 comprend, en outre, un profil des vitesses de vent établi sur le ray-grass avoisinant le champ de betteraves.

Aptères

Le tableau IV donne la répartition des aptères capturés en fonction des distances auxquelles ils ont été retrouvés par rapport aux plantes-sources après 1, 2 et 3 jours de dispersion.

Seuls trois Myzus persicæ ont été retrouvés à des distances inférieures à 5 m, 3 jours après le lâcher. En ce qui concerne Aphis fabæ, deux individus ont été capturés respectivement à 6 et 7 m de leur point de départ, 1 jour après le lâcher, deux autres ont été pris à 14 et 21 m après 2 jours de dispersion, enfin trois autres aptères ont été localisés à 6, 13 et 14 m au bout de 3 jours.

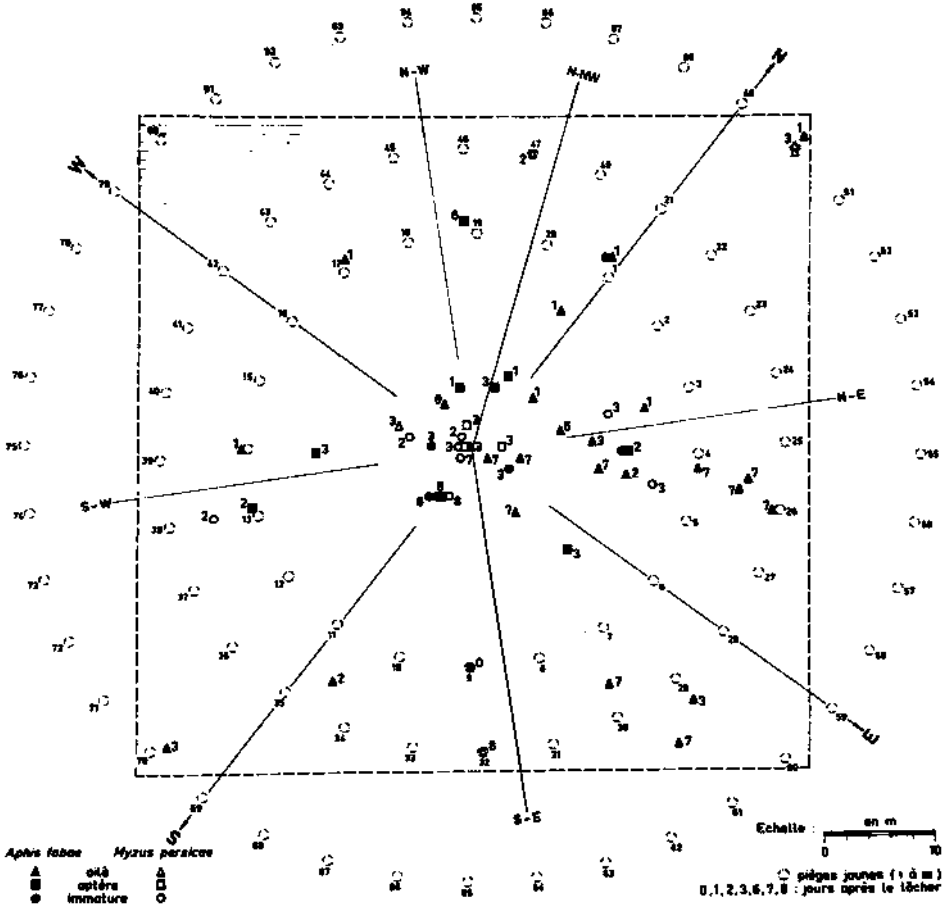

FIG. 4. Essais de 1965: schéma général de dispersion des aphides marqués capturés.

TABLEAU III. OBSERVATIONS METEOROLOGIQUES FAITES A GEMBLOUX (21-31 JUILLET 1965)

Radiation solaire (h)		Précipitations (mm)		Humidité relative [a] (%)	Température moyenne sous-abri [a] (°C)
Heures observées	Moyenne 1945-59 (21-31 juil.)	Hauteur observée	Moyenne 1945-59 (1-31 juil.)		
44,25	70,52	71,6	81,0	77,9	15,7

[a] Moyenne de trois observations faites à 8, 14 et 17 h.

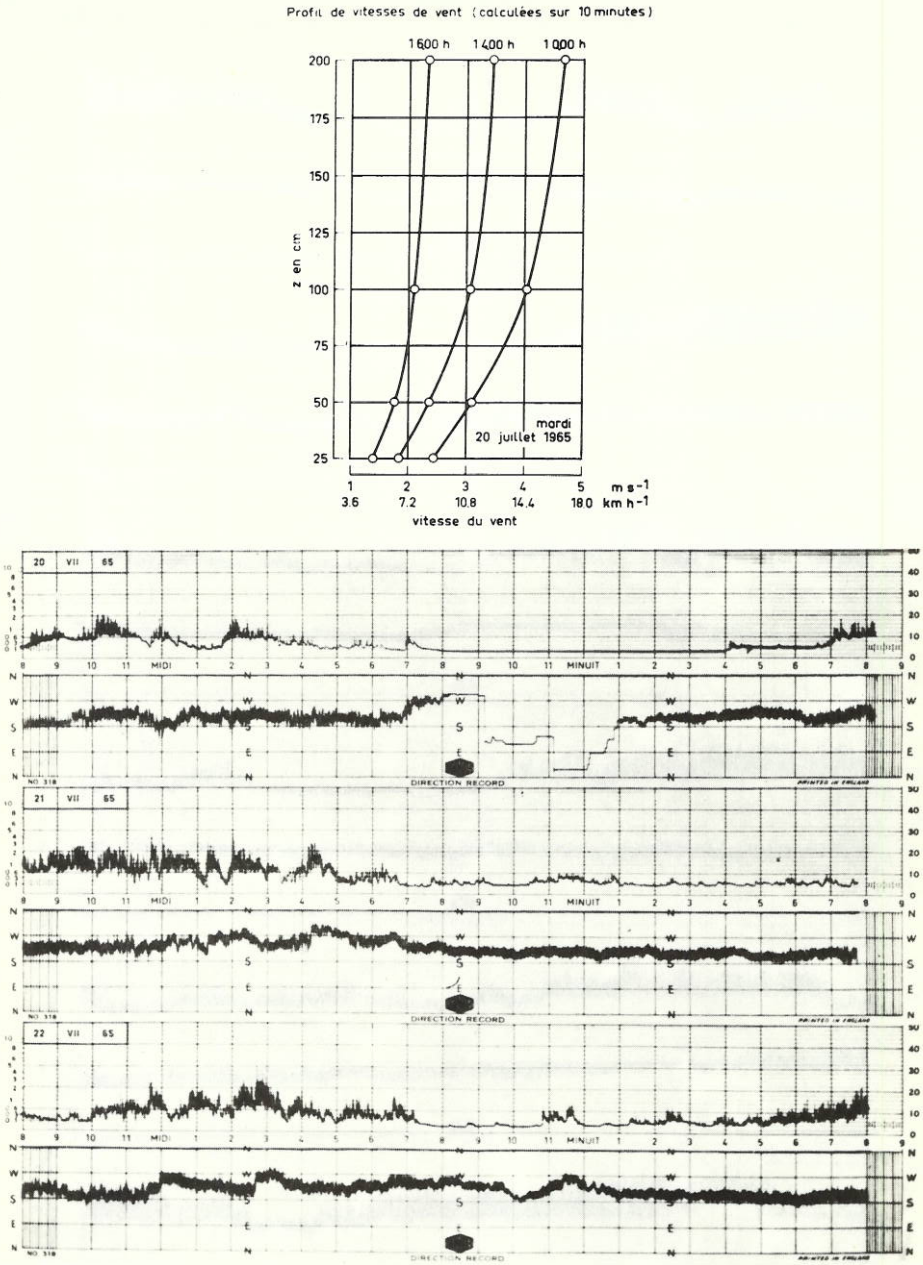

FIG. 5. Essais de 1965 : anémogrammes des 20, 21 et 22 juillet et profil des vitesses de vent du 20 juillet.

Les conditions climatiques défavorables dans lesquelles ont été effectuées les captures n'ont pas permis de retrouver un plus grand nombre d'individus. La capture de 12 aptères au cours de cet essai

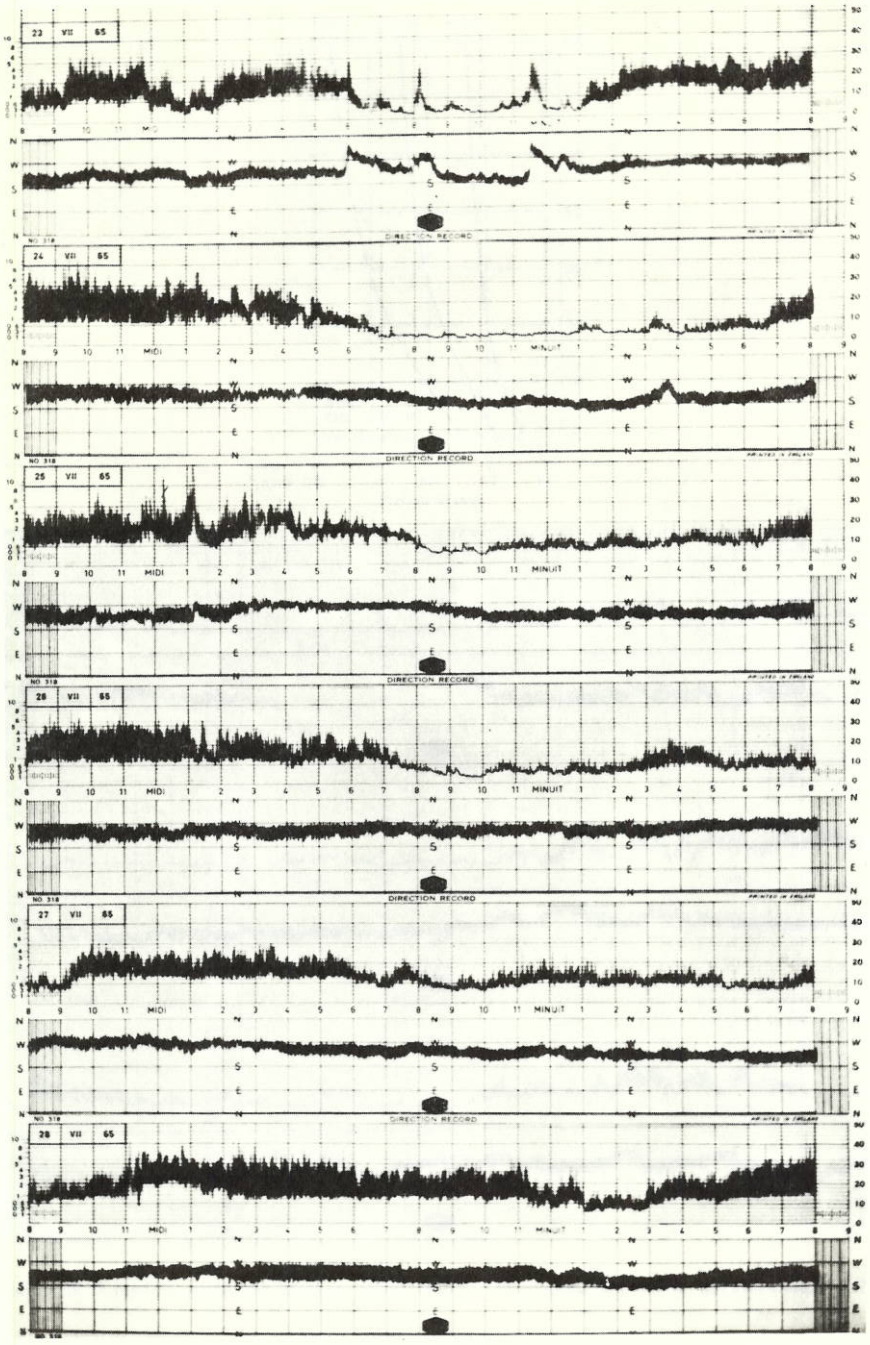

FIG. 6. Essais de 1965 : anémogrammes des 23-28 juillet.

TABLEAU IV. REPARTITION DES APTERES MARQUES CAPTURES APRES 1, 2 ET 3 JOURS DE DISPERSION EN FONCTION DE L'ELOIGNEMENT PAR RAPPORT AUX PLANTES-SOURCES
Essais de 1965

Distance (m)	Myzus persicæ			Aphis fabæ		
	1 jour	2 jours	3 jours	1 jour	2 jours	3 jours
0 - 5	-	-	3	-	-	-
5 - 10	-	-	-	2	-	1
10 - 15	-	-	-	-	1	2
> 15	-	-	-	-	1	-

TABLEAU V. REPARTITION DES AILES MARQUES EN FONCTION DE L'ELOIGNEMENT PAR RAPPORT AUX PLANTES-SOURCES

Distance (m)	Myzus persicæ							Aphis fabæ						
	Jour de capture							Jour de capture						
	0	1	2	3	6	7	8	0	1	2	3	6	7	8
0 - 5	-	-	-	-	-	-	-	-	-	-	1	-	2	1
5 - 10	-	-	-	1	-	-	-	-	1	-	-	-	1	-
10 - 15	-	-	-	-	-	-	-	-	-	1	1	-	1	-
15 - 20	-	-	-	-	-	-	-	1(P)	2	-	-	-	-	-
20 - 25	-	-	-	-	-	-	-	-	3	1	-	-	4	-
25 - 30	-	-	1(P)	-	-	-	1(P)	-	-	-	-	-	1	-
30 - 35	-	-	-	-	-	-	-	-	-	-	1	-	1	-
35 - 40	-	-	-	2(P)	-	-	-	-	-	-	1	-	-	-
> 40	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Total des ailés marqués	5							25						
Total des captures	276							1017						

P = captures dans un piège jaune.

(9 Aphis fabæ et 3 Myzus persicæ) étaie cependant les observations faites les deux années précédentes relatives à la grande mobilité des deux espèces. En outre, 7 individus (5 Aphis et 2 Myzus) ont été retrouvés en dehors du secteur, des vents ayant soufflé au cours de l'essai.

Ailés

Les captures effectuées du 20 au 28 juillet figurent dans le tableau V. Trente aphides ont été capturés manuellement, 5 Myzus (dont 4 dans les

pièges) et 25 Aphis (dont 1 dans un piège). Parmi les 30 ailés capturés, 10 (3 Myzus et 7 Aphis) l'ont été en dehors du secteur, des vents ayant soufflé au cours de la période envisagée (fig. 4). Rappelons que ces vents ont soufflé régulièrement du sud-ouest, avec des directions extrêmes ne dépassant pas le nord-ouest et le sud-sud-est, si nous ne tenons pas compte du vent très faible qui a soufflé au cours de la nuit du 20 au 21 juillet.

Les ailés retrouvés dans des directions opposées ont probablement volé à faible hauteur, au-dessus des plantes, et pendant les périodes où le vent était relativement calme. L'individu capturé le 20 juillet vers 15 h dans le piège 9 (Aphis fabæ) a été retrouvé au sud-est de la parcelle, le vent ayant soufflé du sud-ouest au sud-est entre le lâcher et la capture, à une vitesse oscillant entre 5 et 20 km/h à 10 m de hauteur. (Le profil des vitesses de vent de la figure 5 a été établi sur un gazon proche du champ de betteraves.)

DISCUSSION

Myzus persicæ a toujours été considéré comme un vecteur très mobile. Cependant, les distances parcourues indiquées par les auteurs sont bien inférieures à celles que nous avons enregistrées. Rappelons que c'est Davies [5] qui a attiré l'attention sur la mobilité des formes aptères de Myzus persicæ dans un champ de pommes de terre et sur leur importance dans la dissémination des viroses. En marquant les individus au bleu d'aniline il put mettre en évidence des déplacements de l'ordre de 7 yards en 24 h. Le fait que Bjorling et al. [11] ne signale que des déplacements limités (8, 40 m en 8 jours) peut s'expliquer par un manque de sensibilité de la méthode autoradiographique de détection. Ribbands [10], en partant d'un nombre connu d'aptères de Myzus persicæ placés au centre d'une parcelle de betteraves sucrières non encore infestée, n'a pu mettre en évidence que des déplacements relativement limités par suite, semble-t-il, de la méthode d'échantillonnage adoptée.

Pour Aphis fabæ, la seule donnée concrète résulte de travaux de Bjorling et al. [11] : 3, 25 m en 8 jours.

Nos essais nous ont permi de mettre en évidence une mobilité beaucoup plus grande, tant chez les aptères d'Aphis fabæ que chez ceux de Myzus persicæ (7 m en 24 h, en moyenne), la possibilité d'un transport par le vent étant exclue. Il semble donc que l'efficacité moindre des aptères d'Aphis fabæ soit due principalement au fait qu'il n'est pas un vecteur de la jaunisse modérée de la betterave et que son efficacité de transmission des autres viroses est moindre.

L'importance des ailés dans la dissémination rapprochée des viroses ne peut cependant être niée. Elle a été supposée, dès 1946, par Broadbent [6]. Ribbands [10] lui-même leur attribue un rôle dans la dispersion rapprochée des viroses de la betterave, bien que leur part d'intervention ne soit pas connue. Shaw [17], étudiant le vol des ailés d'Aphis fabæ, distingue parmi ceux-ci, outre ceux qui effectuent un long vol migratoire avant de se fixer et de se reproduire, des individus qui ne prennent leur vol qu'après avoir déposé un certain nombre de larves et qui ont perdu, de ce fait, une partie de leur énergie migratoire. Ces derniers interviendraient, d'une façon significative, dans la dispersion des viroses à courte distance.

Le caractère actif de l'envol a été étudié par Haine [18], alors que la phase active du vol de Myzus persicæ et d'Aphis fabæ a fait l'objet des recherches de Mueller [19] et de Mueller et Unger [20, 21]. Ces auteurs ont observé des vols actifs, contre des vents pouvant atteindre 2 m/s (7,8 km/h). Malgré la densité relativement faible de nos échantillonnages en 1965, nous avons pu capturer un assez grand nombre d'ailés dans le champ expérimental, dont un tiers se sont déplacés dans des directions opposées aux vents. Nos captures semblent donc souligner le rôle des ailés dans la dispersion rapprochée des viroses et le caractère actif de leur vol.

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REFERENCES

[1] WATSON, M.A., HULL, R., BLENCOWE, J.W., HAMLYN, B.M.G., Ann. appl. Biol. 38 (1951) 743.
[2] BJORLING, K., Acta Agric. scand. 2 (1952) 258.
[3] BJORLING, K., OSSIANNILSSON, F., Socker 14 1 (1958) 1.
[4] HEATHCOTE, G.D., COCKBAIN, A.J., Ann. appl. Biol. 53 (1964) 259.
[5] DAVIES, W.M., Bull. ent. Res. 23 (1932) 535.
[6] BROADBENT, L., Ann. appl. Biol. 33 (1946) 360.
[7] BROADBENT, L., GREGORY, P.H., Ann. appl. Biol. 35 (1948) 395.
[8] BROADBENT, L., Ann. appl. Biol. 37 (1950) 58.
[9] BROADBENT, L., Proc. Conf. Potato Virus Diseases, Lisse, 13-17 Aug. 1951, Laboratorium voor Bloembollenonderzoek te Lisse 94, H. Veenman & Zonen, Wageningen (1951) 9.
[10] RIBBANDS, C.R., Bull. ent. Res. 54 (1953) 267.
[11] BJORLING, K., LIHNELL, D., OSSIANNILSSON, F., Acta Agric. scand. 1 (1951) 301.
[12] WATSON, M.A., NIXON, H.L., Ann. appl. Biol. 40 (1953) 537.
[13] MORTREUIL, M., C.r. Séanc. Soc. Biol. 5 (1959) 755.
[14] BANKS, C.J., NIXON, H.L., Entomologia exp. appl. 2 (1959) 77.
[15] BANKS, C.J., Radioisotopes and Radiation in Entomology (Proc. Sym. Bombay, 1960), IAEA, Vienna (1962) 175.
[16] KLOFT, W., EHRHARDT, P., Radioisotopes and Radiation in Entomology (Proc. Sym. Bombay, 1960), IAEA, Vienna (1962) 181.
[17] SHAW, M.J.P., Rep. Rothamsted exp. Stn for 1966 (1967) 201.
[18] HAINE, E., Nature 175 (1955) 474.
[19] MUELLER, H.J., Z. PflKrankh. PflPath. PflSchutz 69 (1962) 385.
[20] MUELLER, H.J., UNGER, K., Züchter 22 (1952) 206.
[21] MUELLER, H.J., UNGER, K., Forschn Fortschr. 29 (1955) 229.

DISCUSSION

H. MARCHART (Chairman): In dispersal studies one frequently finds a dependence of migratory activity on the population density. Did you observe an effect of this kind in your experiments?

A. RIGA: We were not able to investigate this effect, since we knew neither the population density on the host-plants at the outset of the experiment nor the ratio of individuals of the two species on these plants.

W. J. KLOFT: Overpopulation of host-plants by aphids often results in migration; we observed this causal relation in the laboratory and in the field. In such cases winged as well as wingless aphids, and even larvae, may be observed 'walking' out. Your interesting results clearly demonstrate the great significance of migration in the dispersal of virus-carrying aphids. Our own observations are in close agreement.

A. RIGA: Occurrence of winged aphids in our experiments was probably a consequence of what is called the 'group effect', leading to the migration we observed in the field when the cloth covering the hosts was removed. We did not note whether the winged aphids walked or flew.

EFFECTS OF IRRADIATION ON INSECT HOST-PARASITE RELATIONSHIP

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Abstract

EFFECTS OF IRRADIATION ON INSECT HOST-PARASITE RELATIONSHIP. Effects of host irradiation on the development of its parasite were investigated. Females of Bracon brevicornis readily accepted irradiated larvae of the wax moth (Galleria mellonella) and rice moth (Corcyra cephalonica) for oviposition. However, irradiated wax moth larvae adversely influenced the viability of eggs laid on them and also the survival of the parasite grubs feeding on their bodies. The female grubs were affected more than the males. Rice moth larvae, on the other hand, exerted no significant influence on the viability of parasite eggs, but adversely affected the survival of the grubs. The progeny of parents that had been reared on irradiated larvae also exhibited some developmental changes although grown on non-irradiated host larvae, and these changes were more pronounced when G. mellonella was used as the host insect.

1. INTRODUCTION

Accelerated mass production of entomophagous insects is often necessary for inundation releases to correct any upset in the natural parasite-host balance. Propagation of entomophagous insects involves three closely interrelated organisms: the entomophagous species, its host, and the host-plant or food substrate. The success of a breeding programme is therefore a matter of synchronizing three life cycles, i. e. the plant, the phytophagous insect and the entomophagous species. In the propagation of an ectoparasite, a continuous supply of the particular developmental stage of the host insect required for parasite development is sometimes difficult to maintain because of the inherent biological peculiarities of the host insect.

Radiations were shown to prolong the development of insects, to inhibit pupation and to increase adult longevity [1-4]. One could take advantage of these facts to ensure a continued supply of the host for mass multiplication of the parasite. However, it was thought pertinent to ascertain the influence of the irradiated host on the development of its parasite. Investigations were therefore carried out on the development of a braconid parasite on two different hosts.

2. MATERIALS AND METHODS

A braconid parasite, Bracon brevicornis (Hymenoptera), and its two lepidopterous laboratory hosts, the rice moth (Corcyra cephalonica) and the wax moth (Galleria mellonella), were taken from laboratory cultures and utilized in these investigations. The larvae of the rice moths were reared on crushed sorghum containing 5% yeast and those of the wax

moths on a medium containing four parts each of broken wheat and wheat bran and one part each of yeast, milk powder, honey and glycerine.

To study the development of the parasite on irradiated host larvae, ten pairs of freshly emerged male and female adult parasites were caged in a glass jar and provided with 30 wax moth larvae which had previously been subjected to a gamma irradiation dose of 40 krad. A second group of ten pairs was supplied with unirradiated host larvae for parasitization and served as control. After a lapse of 24 hours, the parasitized larvae were removed and the parasite eggs collected, counted and replaced on the same host larvae for further development. The parasite grubs fed on the body fluids of the host larvae and, when fully grown, pupated. The sex of the adults was determined on their emergence. From these adults ten pairs were selected in random fashion and kept singly in glass vials, each of which contained two drops of 10% honey-water for use as food. Each pair was provided daily with four unirradiated host larvae for oviposition. Observations on the daily egg laying by each female and the subsequent development of the eggs to the adult stage were recorded. In another experiment the same procedures were repeated with the larvae of C. cephalonica as the host insect.

3. RESULTS

When the females were allowed to parasitize wax moth larvae, no significant difference in their acceptance of irradiated and non-irradiated host larvae was observed. However, the viability of the eggs laid on irradiated hosts seemed adversely affected (Table I). The grubs feeding on irradiated host larvae were similarly influenced, only 55.3% successfully completing their development up to the adult stage, in contrast to 73.03% of the grubs grown on normal hosts. This difference in growth characteristics on the two host media was statistically significant. The sex ratio (male : female) of adults which developed from grubs feeding on irradiated larvae was also significantly reduced as compared with that of the control insects.

TABLE I. DEVELOPMENT OF B. brevicornis WITH G. mellonella AS HOST INSECT

	Irradiated host	Non-irradiated host
Total number of eggs laid by 10 parasite females in 24 hours	974	935
Per cent viability of eggs	66.83	75.43
Per cent larvae becoming adults	55.30	73.03
Sex-ratio (male: female)	1: 0.69	1: 1.106

TABLE II. DEVELOPMENTAL CHARACTERISTICS OF THE PROGENY OF PARENTS REARED ON G. mellonella LARVAE

	Parents reared on	
	Irradiated host	Non-irradiated host
Fecundity	629.9	698.9
Per cent successful completion of development from egg to adult	63.9	66.06
Sex-ratio (male: female)	1: 0.299	1: 0.5665

TABLE III. DEVELOPMENT OF B. brevicornis WITH C. cephalonica AS HOST INSECT

	Irradiated host	Non-irradiated host
Total number of eggs laid by 10 parasite females in 24 hours	274	323
Per cent eggs viable	86.12	84.84
Per cent larvae becoming adults	77.56	95.61
Sex-ratio (male: female)	1: 1.011	1: 1.063

Progeny of parents reared on irradiated larvae also exhibited some developmental changes although grown on normal host larvae (Table II). This was evident from the fact that only 63.9% of the eggs laid by females reared on irradiated host larvae developed into adults, whereas 66.06% of the eggs from females reared on normal host larvae completed development into the adult stage. A preponderance of males in the progeny of parents reared on irradiated hosts was also observed.

Unlike G. mellonella, the irradiated larvae of C. cephalonica exerted no influence on the viability of the eggs laid on them by normal females (Table III). However, the parasite grubs feeding on the body fluids of irradiated larvae showed considerable mortality. The reduction in the number of grubs successfully developing on irradiated hosts was statistically significant when compared with the number developing on normal host larvae. The progeny of parents reared on irradiated larvae of C. cephalonica showed no developmental abnormalities (Table IV).

4. DISCUSSION

The adverse effect on the development of the parasite reared on irradiated wax moth larvae indicated a change in the suitability of the

TABLE IV. DEVELOPMENTAL CHARACTERISTICS OF THE PROGENY OF PARENTS REARED ON C. cephalonica LARVAE

	Parents reared on	
	Irradiated host	Non-irradiated host
Fecundity	493.6	425.1
Per cent successful completion of development from egg to adult	64.03	62.38
Sex-ratio (male: female)	1: 0.4158	1: 0.3923

host insect. Physiological changes in insects following irradiation were reported by many workers. Differences in the haemolymph protein fraction between irradiated and control Ephestia larvae were observed [5]. Kritskii and Chiwei [6] reported a considerable fall in adenosine triphosphate content and activation of autolysis immediately following irradiation of the bee moth (Gallfria mellonella) larvae with 2 krad. A delay in pupation of X-irradiated Drosphila melanogaster larvae was noticed by Bourgin et al. [7], who suggested that the radiation effect was an abnormal phase between normal and actual pupation, and during this phase a loss in the weight of the larvae with a greatly reduced growth rate of fat bodies took place.

The effect of radiation induced physiological changes in the host was more pronounced on the post-embryonic development of the parasite. The significant decrease in the male-to-female ratio amongst adults developing on irradiated host larvae (G. mellonella) indicated that the female parasite grubs suffered greater mortality while feeding on body fluids of the irradiated host larvae. This could be the result of differential nutritional requirements, both qualitative and quantitative, of the male and the female parasite grubs. No experimental evidence in support of the existence of such a nutritional dimorphism was found in the radiation literature. However, the existence of such a phenomenon was demonstrated in parasitic Hymenoptera. In Coccophagus cowperi the host species supporting development of male and female grubs are different [8]. In Encarsia spp., eggs developing into males were deposited endoparasitically in lepidopterous hosts, and those developing into females in homopterous hosts [9].

The physiological changes in the host not only affect the development of the parasite, but also that of the progeny, even when the latter are allowed to develop on normal hosts. This adverse effect on the progeny could be the result of inadequate nutrition of the parents. Flanders [10], while studying the effects of cold storage on the parasitic Hymenoptera, observed that: "the metabolic activity during the mature larval or pupal periods is dependent on nutritive materials accumulated in the body during the immature larval stages. The organs most likely to be affected by insufficient nutrition are those used in reproduction, with the result that the germ cells are adversely affected, and the spermatozoa in

particular lack vitality." In B. brevicornis unfertilized eggs develop into males and fertilized ones into females. The preponderance of males in the progeny of parents reared on irradiated host larvae could be due to the poor vitality of sperms, with the resulting reduction in the number of fertilized eggs laid.

The developmental changes suffered by the parasite growing on irradiated rice moth larvae were less pronounced compared with those occurring when wax moth larvae were used as hosts. This could be attributed to the differential radiosensitivity of these two host species. A dose of 15 krad prevented pupation of the wax moth larvae, whereas a dose of 40 krad was required to produce the same degree of inhibition in the case of the rice moth (Rahalkar, unpublished data). Whiting [11] oberved no mutagenic or lethal effects on the parasite Habrobracon reared on heavily irradiated Ephestia larvae. This could be due to the higher tolerance of this insect to radiation. When full-grown E. kuhniella larvae were X-irradiated with doses ranging from 40 to 160 krad, 23.16% of the larvae were alive 30 to 40 days after irradiation [12]. It was concluded that irradiation of the host insect greatly influences the development of the parasite and also that of its progeny in such a manner that irradiation of a host insect is of doubtful value in modifying the development of the host insect to suit the requirements of the parasite.

REFERENCES

[1] ABDEL-MALEK, A.S., Bull. ent. Res. 52 4(1961) 701.
[2] ERDMAN, H.E., Int. J. Radiat. Biol. 5 4 (1962) 331.
[3] DAY, M.F., OSTLER, I.I., Insect Pathology – an advanced treatise, Vol. 1, ch. 2: Physical injuries (Steinhaus, E.A., Ed.) Academic Press, New York (1963).
[4] BALOCK, J.W., Jr., BURDITT, A.K., CHRISTENSON, L.D., J. econ. Ent. 56 1(1963) 42.
[5] ERDMAN, H.E., Research and development in progress, Biology and Medicine, Issue No. 2, TID-4201, AEC (Nov. 1963) 127.
[6] KRITSKII, G.A., CHIWEI, C., Biochemistry USSR, 26 (1961) 224.
[7] BOURGIN, R.C., KRUMINS, R., QUASTLER, H., Radiat. Res. 5 6 (1956) 657.
[8] FLANDERS, S.E., Univ. Calif. Publs Ent. 6 (1937) 401.
[9] FLANDERS, S.E., Entomologia exp. appl. 2 (1959) 125.
[10] FLANDERS, S.E., J. econ. Ent. 31 5 (1938) 633.
[11] WHITING, A.R., Anat. Rec. 111 (1951) 565.
[12] WHITING, A.R., Anat. Rec. 108 (1950) 609.

DISCUSSION

V. LABEYRIE: Do not the differences in your results for the wax moth (G. mellonella) and the rice moth (C. cephalonica) arise from the fact that in unirradiated mellonella the embryonic mortality is already higher than in unirradiated cephalonica, so that it could be assumed that while cephalonica is a favourable host, mellonella is not, and any further deterioration in the conditions has fatal results?

G. W. RAHALKAR: The wax moth and the rice moth appear to be equally suitable hosts for the development of this parasite. The difference in percentage viability of eggs laid on unirradiated larvae of these two moths can be ascribed to natural variation influenced by environmental

factors. The two main experiments were performed at different times and these two controls cannot therefore be directly compared.

V. LABEYRIE: Is the variation greater for isolated females on irradiated larvae?

G. W. RAHALKAR: This variation is not significantly large.

P. PELEGRIN: Does the 40-krad dose given to the host cause its death or simply arrest its development?

G. W. RAHALKAR: In the case of the rice moth, pupation was inhibited above doses of 40 krad, but irradiated larvae survived for a considerable period. In the case of the wax moth the larval mortality curve climbs rapidly.

INVASION OF CONIFER PLANTATIONS BY RADIOACTIVELY LABELLED *Hylobius abietis* L.

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Abstract

INVASION OF CONIFER PLANTATIONS BY RADIOACTIVELY LABELLED *Hylobius abietis* L. *Hylobius abietis* L. weevils, radioactively labelled by dipping into solutions containing ^{46}Sc, were released around two model areas representing recently cleared cutting areas freshly planted with Norway spruce. Half of the weevils released around one model area were also labelled with different colour marks. The invasion, of both the natural *Hylobius* population and the released labelled weevils, was studied by making repeated inspections of attractant pine billets evenly distributed over the experimental areas. At certain intervals the spruce plants on the areas were inspected for feeding marks to evaluate the progress of the attack.

The greatest number of labelled weevils, amounting to 18% of those released, was found in one experimental area one day after release. In this area, successively diminishing numbers of weevils found at the billets (both labelled weevils and natural population) and an increasing degree of feeding on the plants indicated that the weevils did not leave the plantation but changed their behaviour. The weevils often dug into the soil, which was proved by measurements of ground radioactivity at billets and stumps. In the other area, which had richer vegetation, only small changes in numbers occurred.

In all inspections, most of the labelled weevils were found near the border of the experimental area, but the percentage of weevils in the middle of the area increased slowly. One day after release the greatest distance a radioactively-plus-colour-labelled beetle had moved into the area was 34 m, while the average distance was only 4.15 m. There were no significant differences in the speed of invasion from different directions into the plantation. From the results and observations it can be concluded that most or all released weevils invaded the plantations by crawling, and not by flying.

The natural populations invaded one experimental area slowly from the borders, and the other area faster and more evenly distributed. Accordingly, the distribution of damage by feeding was uneven in one area and comparatively even in the other.

One practical consequence of this study is the recommendation that plant protection measures be concentrated on the borders of conifer plantations. Even plants in the central area of plantations should be protected. Concentrations of weevil-attracting materials, such as attractant insecticide traps, should be avoided in the centres of plantations.

1. INTRODUCTION

The weevil, *Hylobius abietis* L., causes considerable damage in European conifer plantations by feeding on the young plants. There are various methods of preventing such damage: keeping the weevils away from the plantations (reforestation several years after clear cutting, diminution of beetle population, trap ditches), protecting the plants by insecticides (treatment before planting or in the plantations) or controlling the weevils in the plantations (traps, insecticide-treated billets, total insecticide treatment of plantation). For more than 15 years synthetic insecticides have been used successfully; nevertheless the problem of *Hylobius* control is not completely solved and requires greater knowledge of the ecology and the behaviour of the species.

Young conifer plants on fresh clear-cutting areas where there are stumps and other remains of conifer trees are particularly susceptible to attack by Hylobius. These plantations are invaded in spring by Hylobius weevils from the vicinity, which feed on the plants and lay their eggs on the stumps. The invasion of weevils into the plantations and the distribution of their feeding in the plantation have hitherto been studied systematically only to a limited degree. Invasion and feeding, however, are closely connected with the problem of control.

Temperature, humidity (of air and soil) and vegetation influence the appearance of the weevils, their activities and feeding [1-3]. Schmidt [3] studied the migrations in experiments with fed, colour-marked weevils. The beetles can fly rather long distances, but, on the other hand, the trap ditch control method is based on the fact that the weevils often invade the plantations by crawling on the ground. They prefer to stay under fresh conifer bark, but later leave it [4] and enter the soil for oviposition.

In 1966 and 1967 we conducted experiments to clarify the answers to the following questions:

In what way do weevils entering a plantation from its borders disperse over the area, and what is the time sequence of the invasion?

Do the weevils prefer certain directions of invasion?

Are there differences between the manner of invasion of beetles starting close to the area and that of beetles coming from a greater distance?

When and to what extent do the weevils enter the soil?

What is the time sequence and the spatial distribution of feeding on the plants?

The beetles were released at the borders of the experimental areas and were labelled so that they could be distinguished from the natural Hylobius population. For several reasons, labelling with a γ-ray emitting radioisotope was chosen: fast and easy labelling, no effect on the behaviour of the insects, easy detection, and detection of weevils under the soil surface. The experimental areas were laid out as model areas, because conditions in plantations on natural clear-cutting areas usually vary in the distribution of stumps and vegetation in such a manner that it is difficult to analyse and generalize the results.

2. EXPERIMENTAL METHODS

2.1. Experimental areas

The experimental areas were rectangles measuring 80 m × 96 m. They were situated in forest districts in central Sweden and were surrounded on three sides by uniform forests. No conifer trees had been felled within a distance of at least 2 km from the areas for more than one year before the experiments. This eliminated other sources of attraction for the weevils. The areas were farm land with uniform weed vegetation. The experimental area used in 1966 (Värmland) had moist clayey soil with thick weed vegetation; the experimental area in 1967 (Östergötland) had dry sandy soil with poor vegetation. The areas were

planted with Norway spruce in spaces of 2 m × 2 m and 50-cm freshly cut pine billets were laid out on the ground in spaces of 4 m × 4 m. Cuttings of pine trunks, 50 cm long and 12-15 cm in diameter, were put vertically into the ground in spaces of 8 m × 8 m. They protruded 15 cm above the surface and represented artificial stumps.

In this way every model area of 0.77 ha had 120 stumps, 480 pine billets and 1920 spruce plants evenly distributed over the area.

2.2. Labelling and release of weevils

The weevils were collected in sawmills at the beginning of June and kept cool and without food until labelling.

In preliminary experiments with ^{60}Co solution (2.5 μCi/ml) weevils were labelled by dipping them into the solution and by feeding them on pine bark treated with radioactive solution. After several weeks in cages, both groups of weevils behaved normally and were sufficiently radioactive. After being rinsed thoroughly in water, the dipped weevils lost more radioactivity than those labelled with radioactive food. Nevertheless, the dipping method was chosen for the field experiments, because the released weevils were to be hungry.

Because of its shorter half-life ^{46}Sc was used instead of ^{60}Co. The dipping solutions contained $^{46}ScCl_3$, with H_2O and 20% alcohol as a wetting agent. The specific activity was 14.4 μCi/ml in the 1966 experiment and 44.8 μCi/ml in 1967. Dipping was performed in the open air near the experimental areas. The dipping solution was in a pit in the ground. The operator was shielded by a soil embankment and lead bricks, viewed the solution in a mirror and manipulated with rods and clamps. Small, perforated PVC bottles, each containing 88 weevils, were dipped into the radioactive solution and then put aside to dry. Each bottle was used only once. On both 11 June 1966 and 13 June 1967, 3872 weevils were labelled radioactively and released at equal distances on the borders of the experimental areas. Dipping and release took less than four hours.

1760 of the weevils released in 1967 were labelled with different colours on the elytra before radioactive labelling. Four groups of 440 weevils each were labelled with four different colours and released on each of the four sides of the experimental area. Thus each side had Hylobius with a certain colour. In three cages near the experimental area used in 1967, untreated, radioactively labelled and radioactively-plus-colour-labelled weevils were kept for comparison.

2.3. Inspections

The experiments were inspected at intervals until August, the purpose of the inspections being to examine the presence of labelled and unlabelled weevils and of radioactivity at the pine billets and artificial stumps. The radioactivity was determined with a scintillation counter and GM counters. Random samples were taken inside and outside the area to determine the presence of weevils and radioactivity on the plants. The extent of feeding on every plant was rated from 0 to 5.

In later inspections the damage by feeding and the breeding on the stumps were studied.

3. RESULTS

3.1. Radioactivity and survival of weevils

In the 1967 experiment the radioactivity of one weevil one day after labelling was, on the average, about 0.1 μCi. It decreased rather rapidly, and five days after labelling amounted to only about 50%, and after 2 months (caged weevils) about 7%. In the 1966 experiment the radioactivity was lower than in 1967.

The decrease of radioactivity was caused by the radioactive decay of ^{46}Sc and by the radioactive layer being rubbed off the bodies of the insects. The importance of rubbing off was proved by two results of the experiments:

(1) The soil under the pine billets and at the stumps often showed considerable radioactivity (see section 3.2).
(2) In the cages 2 months after labelling the dead weevils had a significantly higher radioactivity than the live Hylobius.

No obvious differences between the degree of survival of untreated, radioactively-labelled weevils and that of radioactively-plus-colour-labelled weevils in the cages were observed. In the experimental area, there was no great difference between the presence of radioactively-labelled weevils and that of radioactively-plus-colour-labelled weevils.

3.2. Time sequence of weevil appearance

In both experiments radioactivity in the soil was already observed one day after release of the weevils. It was thus apparent that the weevils soon entered the soil, also at the stumps. The number of places with radioactivity in the soil gradually increased. Radioactivity in the soil does not imply the presence of a labelled weevil; in many places examined no beetles were found. The weevils had apparently left these places, but lost some of the radioactive layer on their bodies by friction with soil particles (see section 3.1).

The number of labelled weevils found in one inspection in the 1966 experiment did not exceed 0.7% of the beetles released. Therefore, the time sequence for the weevil appearance could not be analysed. In the 1967 experiment, however, 18% of the labelled weevils were found in the experimental area one day after their release. This was the highest number of labelled weevils found. The number of radioactive weevils found in this area later diminished successively (Fig.1).

The unlabelled weevils of the natural population invading the experimental area in 1967 reached their greatest number at the billets before the release of labelled weevils. They then decreased at a rate resembling that of the labelled ones (Fig.1). It may thus be concluded that labelled and unlabelled weevils behaved in the same way. The decrease of weevils found at the billets does not mean that the beetles left the area. The continuous increase of feeding in the area (see section 3.4 and Fig.2) refutes this; a change in the behaviour of the weevils is more probable.

In the 1966 experiments there was no such change in behaviour. The number of unlabelled weevils varied, but irregularly and without the

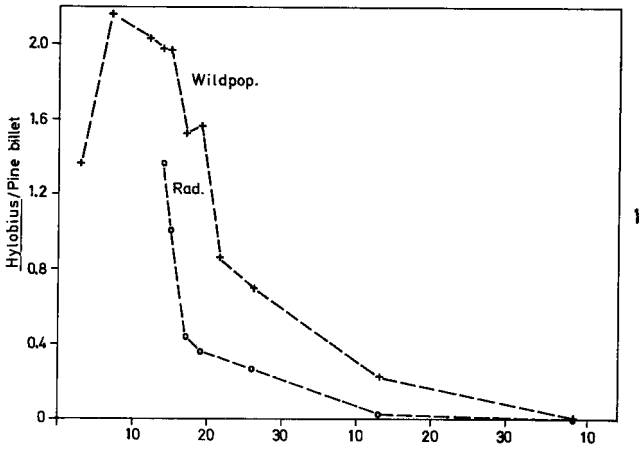

FIG. 1. Experimental area, 1967: mean number of unlabelled (Wildpop) and labelled (Rad) Hylobius weevils per pine billet at different times during the season.

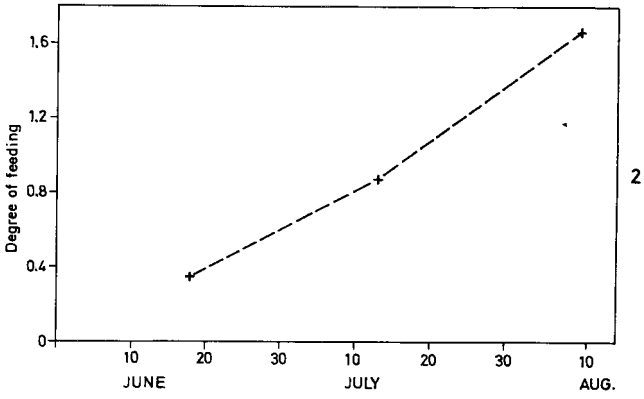

FIG. 2. Experimental area, 1967: mean degree of feeding per spruce plant at different times during the season.

marked decrease shown in Fig. 1. This is seen from the following extract from the protocols:

Date of inspection	16.6.66.	19.6.66.	22.6.66.	27.7.66
Number Hylobius/billet	0.29	0.20	0.41	0.23

In 1966 the maximum number of unlabelled weevils found in one inspection was considerably lower than in 1967.

The different behaviour of the weevils was probably connected with differences in soil, vegetation, and local and microclimate between the two experimental areas.

3.3. Spatial distribution of weevils

The labelled weevils began to invade the experimental areas shortly after release. Single weevils soon reached the middle of the area, but

the majority advanced slowly. In all inspections, considerably more radioactive Hylobius were found in the outer regions of the experimental areas than in the centre. Later, however, there was an increase in the proportion of labelled weevils that advanced further into the areas. The examples in Figs 3-5 illustrate this progress.

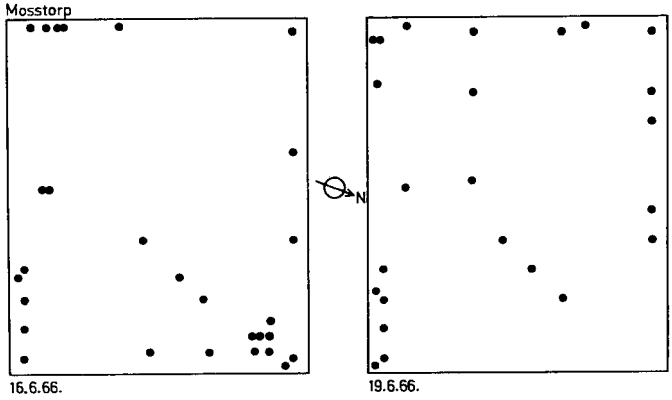

FIG. 3. Experimental area, 1966: spatial distribution of labelled weevils on 16 June (left) and 19 June (right).

FIG. 4. Experimental area, 1967: spatial distribution of labelled weevils on 14 June.

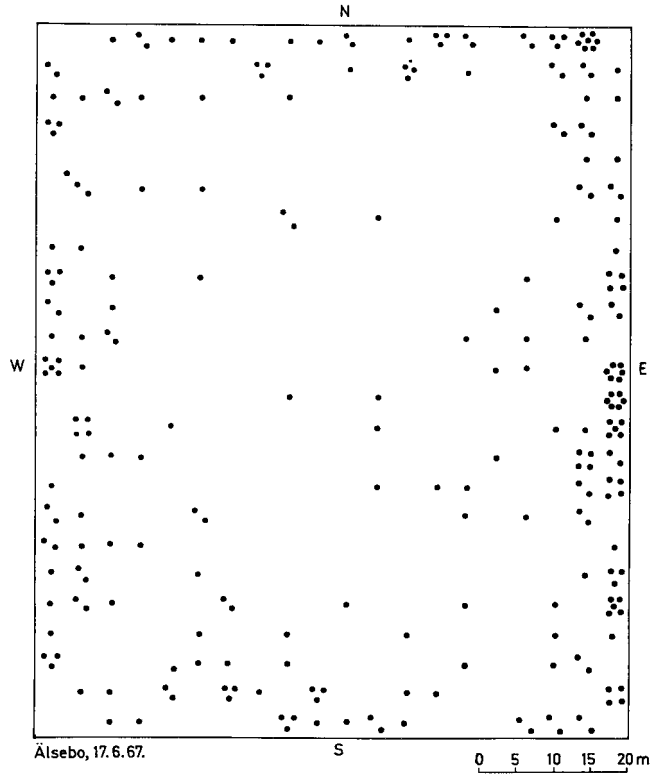

FIG. 5. Experimental area, 1967: spatial distribution of labelled weevils on 17 June.

The radioactively-plus-colour-labelled weevils in the 1967 experiment gave more exact information on the speed of invasion. For every colour-labelled Hylobius found in the area, the shortest distance to the line of release for the respective colour was determined. One day after release one weevil had already advanced at least 34 m. The greatest distance was 90 m (13 days after release). On the other hand, the mean minimum distance increased with time, but was considerably lower (Fig. 6). The speed and distance of invasion of colour-labelled weevils was about the same for the different directions of invasion.

The unlabelled weevils of the two natural populations differed, not only as regards the time sequence (see section 3.2), but also in their spatial distribution in the two experimental areas. In the 1966 experiment, the inspections made in June revealed many more beetles in the outer regions than in the centre of the area. At the end of July, however, there were no longer any distinct differences between the outer and inner regions (Fig. 7). In the 1967 experiment, the unlabelled weevils were relatively evenly distributed over the area.

3.4. Progress and distribution of feeding

In both experimental areas, the mean degree of feeding per plant increased after the middle of June and continued to increase until the

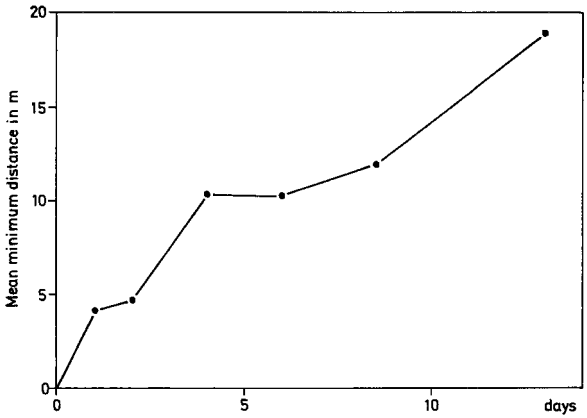

FIG. 6. Experimental area, 1967: mean minimum distance (in metres) covered by radioactively-plus-colour-labelled weevils at different times after release.

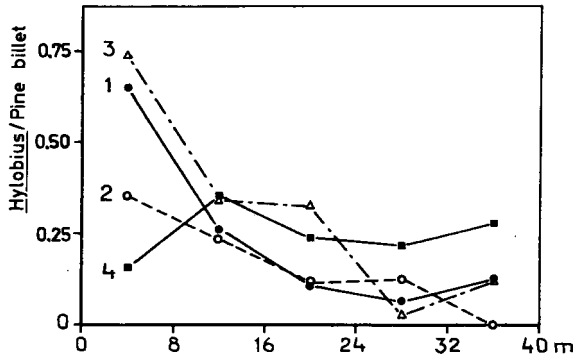

FIG. 7. Experimental area, 1966: mean number of unlabelled weevils per billet at different distances from the border of the area.
4 inspection dates: 1 = 16 June 3 = 22 June
2 = 19 June 4 = 27 July

beginning of August (Fig. 2). Both the number of attacked plants and the extent of feeding on the single plants increased.

As a result of the differences in spatial distribution of the weevils (section 3.3), there were also differences in the spatial distribution of feeding between the two experimental areas. In the 1966 experimental area, the degree of feeding decreased with increasing distance (Fig.8). Thus the later, more random distribution of weevils did not result in a random distribution of feeding damage towards the end of the season. In the 1967 experimental area, however, the plants in the outer parts were somewhat more heavily attacked than those in the centre, but only by the middle of June. The outer parts had a higher percentage of attacked plants and a higher mean degree of feeding per plant. Later in the season, feeding was distributed at random.

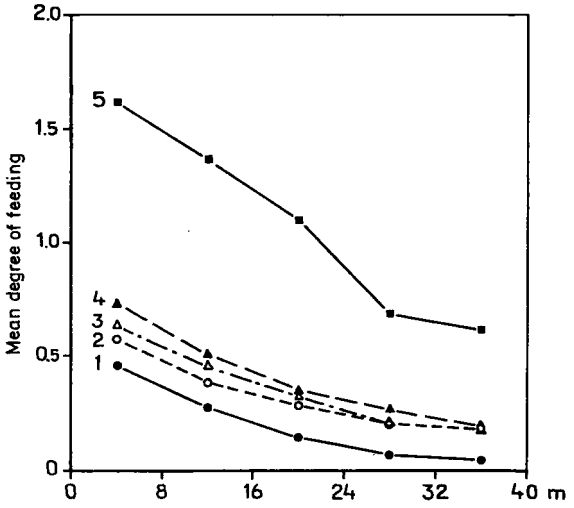

FIG. 8. Experimental area, 1966: Mean degree of feeding per spruce plant at different distances from the border of the area.
5 inspection dates: 1 = 13/14 June 4 = 22 June
2 = 17 June 5 = 28 July
3 = 20 June

3.5. Spatial distribution of Hylobius larvae

In the autumn of 1966 the mean number of Hylobius larvae for the whole area was observed to be 20.8 larvae per stump. Neither differences in the number of larvae in different parts of the area nor differences in the speed of development were noted. Thus either the weevils dispersed evenly over the area for oviposition or – more probably – the limited number of breeding places resulted in complete utilization of the stumps irrespective of differences in density within the weevil population. The inspection of stumps in the 1967 experimental area has not yet been concluded, but it is not expected that differences will occur.

4. DISCUSSION

The course of Hylobius invasions and the distribution of weevils and feeding damage depend on local conditions such as the surroundings of the plantation, soil, vegetation, and the local and microclimate. This was demonstrated by the experiments, which answered the questions listed in the introduction.

Weevils close to the borders of a plantation invaded the area slowly and were for some time unevenly distributed in the plantation. During this time the density of the weevils decreased from the borders to the centre. Apparently the insects moved mainly or exclusively by crawling.

These weevils obviously did not prefer certain directions of invasion occasioned by compass direction, prevailing wind or the slope of the ground.

Weevils arriving at a plantation from a greater distance might invade the area slowly in the same way as weevils starting close to the borders. On the other hand, they might invade the area rapidly and in a short time disperse at random. It is an open question whether such rapidly invading weevils also moved by crawling or whether they flew into the area.

The weevils entered the soil at any time and could leave the soil to move to other places.

Feeding began (in central Sweden) in June and continued until August. The absence of weevils at attracting billets did not imply that they had left the area. They were capable of changing their behaviour and choosing other places.

The spatial distribution of feeding depended on the distribution of weevils. Plantations attacked by slowly invading weevils suffered the heaviest feeding damage in the outer areas. If the weevils invaded rapidly, the feeding was more randomly distributed in the area.

The following practical conclusions were drawn from the results:

Control measures to protect conifer plantations against Hylobius attack should be concentrated in the outer areas of the plantations.

Plants in the centre of plantations also need protection, because weevils might invade the area rapidly and cannot be stopped near the border.

Concentrations of weevil-attracting material in the inner regions of plantations should be avoided.

Attractant weevil traps in the inner parts of plantations would be unsatisfactory as the sole means of control.

REFERENCES

[1] BUTOVITSCH, V., Das Flugvermögen des grossen braunen Rüsselkäfers. Forstwiss. ZentlBl. 54 (1932) 446-60.

[2] FISCHER, K.R., Beiträge zur Ernährungsbiologie von Hylobius abietis L. und Untersuchungen über die Ökologie und Klimatologie seines Nahrungsraumes. Z. angew. Ent. 19 (1932) 250-77.

[3] SCHMIDT, W., Rüsselkäfer - Bilanz, Berlin (1934).

[4] NOVÁK, V., TEMMLOVÁ, B., Přemnožení klikoroha borového (Hylobius abietis L.) v ČSSR a rozbor současných obranných a ochranných opatření, Lesn. Čas. 10 (1964) 659-78.

DISCUSSION

W.F. BALDWIN: I should like to mention that in 1958 Sullivan et al. in Canada reported on a one-year field study of white pine weevils labelled with ^{60}Co. In contrast to the present study, the insects were labelled with 75 μCi of ^{60}Co, and the authors found that such a dose had no significant effect on the degree of survival.

H.H. EIDMANN: Unfortunately, security regulations in Sweden do not allow us to use these high activities in the field.

B

RADIATION EFFECT STUDIES: NON-GENETIC

(Sessions III and IV)

Chairman: H. NÖTHEL

FEDERAL REPUBLIC OF GERMANY

CORRELATIONS, INTERACTIONS AND DIFFERENCES BETWEEN RADIATION EFFECTS ON LONGEVITY AND NATURAL AGING

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Abstract

CORRELATIONS, INTERACTIONS AND DIFFERENCES BETWEEN RADIATION EFFECTS ON LONGEVITY AND NATURAL AGING. The non-genetic overall radiation response of insects is expressed by the fitness components 'developmental rate' and 'adult lifespan', and is hence related to natural aging.

Based on Drosophila, with additional remarks on other insects, the paper demonstrates that, in the developmental stages, ionizing radiation affects the differentiation processes. Distinct sensitive phases are apparent for the induction of different injuries, as are effective phases during which the damage exhibits its lethal action. With increasing differentiation, the radiation sensitivity decreases. The primary biological damage is, at least in part, some kind of somatic mutation.

A review of adult irradiation response is also based on Drosophila. At least two different types of induced mortality are evident. Type 1 occurs early after irradiation with high doses. It is probably due to central nervous injury and is a common effect in insects, as is indicated by a comparison of various species. Type 2 is a more delayed death at median and low doses. It is of different appearance in various insects, and this heterogeneity is even seen within one species. Thus, sterilizing Drosophila females may result in a considerable prolongation or in a drastic reduction of lifespan.

Experimental evidence is found in Drosophila against the 'induced aging' hypothesis of radiation death. Type 1 mortality increases with increasing age at exposure. The amount of the increase in sensitivity depends indirectly on biological age. Both of these parameters are fixed by genotype, as is the mode of interaction between them. Type 2 mortality follows an initial latent period within an 'induced mortality' period. The latter lasts always the same time, independently of age at irradiation. The latent period, however, is shortened in general with increasing age at exposure. This effect seems to depend on metabolic properties rather than on aging itself, as is indicated especially by a prolongation in early adult life.

1. INTRODUCTION

The biological efficiency of the single organism as well as of a population is expressed by its fitness. Hence, the overall radiation response of living systems may be measured by means of fitness components. The non-genetic radiation effects are restricted, then, to those components of fitness involved in the life cycle of a single specimen. In insects, these are mainly reproductive capacity, developmental rate, and adult lifespan. The reproductive capacity is affected mainly by dominant lethals induced in the germ cells and also by lethal effects on the trophocytes [1-6]. The other two fitness components are related to 'aging'. The developmental rate reflects the preadult differentiation or aging processes in a wider sense. The adult lifespan reflects natural aging sensu stricto. Hence, the radiation effects on both of them, and thus the overall non-genetic radiation responses of insects, are covered by the topic 'correlations, interactions and differences between radiation effects on longevity and natural aging'.

2. RADIATION EFFECTS ON DEVELOPMENTAL STAGES

The sensitivities of the various developmental stages to ionizing radiations are rather simply measured by survival to adulthood. The LD_{50} emergence in Drosophila melanogaster clearly depends on the stage irradiated (Fig. 1). It increases from 400 R during cleavage to more than 40 000 R after exposure of pupae. These differences up to a factor of 100 were described in 1927 by Mavor [13]. They are the same in other insects as in Drosophila. For example, the same sensitivity pattern as described in Fig. 1 was found in Habrobracon [14-16], in Dacus and in Ceratitis species [17] and in Anastrepha [18]. On the other hand, the radiation sensitivity in the various developmental stages of Drosophila is constant if measured by means of a single type of cell, the spermatogonia, and by one effect only, the induction of sex-linked recessive lethals (Fig. 1). Therefore, during embryonic, larval and pupal development, it is not only the radiation sensitivity of the whole organism which decreases, but also that of certain cells or cell groups which, in addition, may change in their importance for the overall radiation response. Hence, there are sensitive phases for the induction of a special insult, and effective phases during which the injury becomes visible when the affected system is needed to complete development.

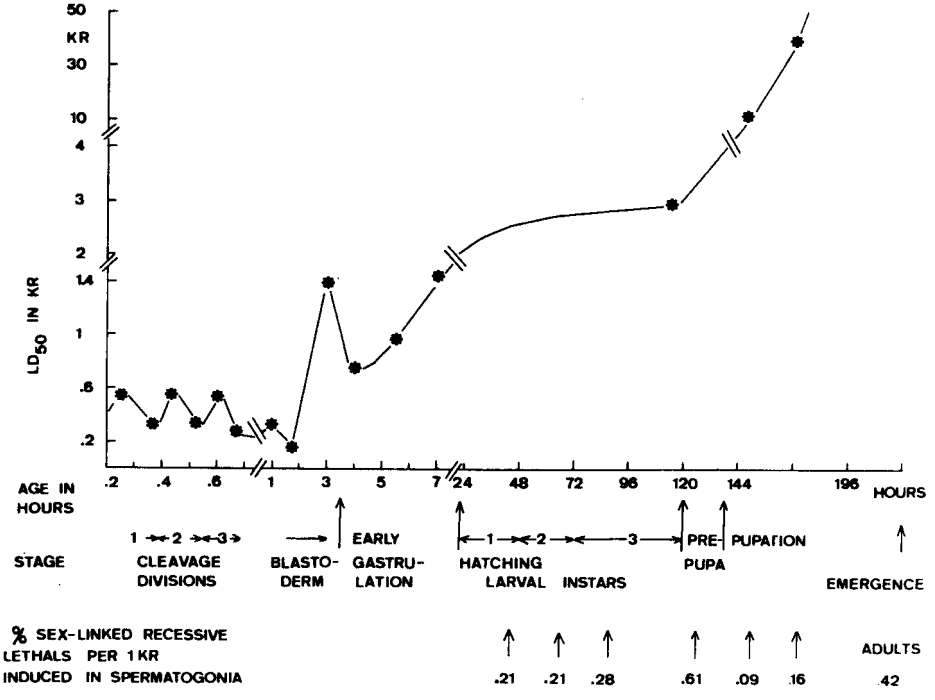

FIG. 1. LD_{50}-emergence after X-irradiation of different developmental stages of Drosophila melanogaster (at 25° C). LD_{50} data after Fritz-Niggli (7-9), Wurgler (10), and own unpublished results; mutation rates after Khishin (11) and (adults) Auerbach (12).

TABLE I. DIFFERENT TYPES OF MORTALITY AFTER X-RAYING 1.75 HOURS OLD EMBRYOS OF DROSOPHILA WITH DIFFERENT DOSES. - Data from FRITZ-NIGGLI (7) and STEENBECK (19)

dose in R	total mortality	frequencies of the different types within total mortality		
		immediate death	early death	late death
8,000	100 %	50 %	50 %	-
400	90 %	-	95 %	5 %
200	65 %	-	60 %	40 %
100	20 %	-	20 %	80 %

Different effective or lethal phases indicate different lethal effects during development. These effects may change with increasing dose. A lethal injury with a late effective phase induced at low doses will be surpassed by another one with an early effective phase induced at the same developmental stage at a higher dose level. Thus, X-irradiating early blastoderm stages of Drosophila resulted in at least three different lethal phases depending on dose (Table I). Of these, the 'immediate death' just after exposure seemed to indicate the complete breakdown of dividing cells. The 'early death' occurred until gastrulation. The interpretation that it was due to the impossibility to initiate gastrulation was confirmed by Fritz-Niggli [8]. Even at the LD_{80} hatching she found no early death after the beginning of pregastrular cell movements at an age of three hours: early type mortality after exposure of 2.5, 3, and over 3-hour-old embryos was 90%, 5% and 0%, respectively. Hence, the sensitive phase for the induction of damage with an effective lethal phase during the early gastrulation ends with the beginning of the latter. In other cases, some time elapsed between the two phases. Thus, the 'late death' was due to damage in the developing larval organs, which predominantly involved the hypodermis, especially that of the mouth region, and the musculature [8, 9]. After the differentiation of larval organs was completed, the sensitive phase for the induction of the above damage ended at 7 hours (Fig. 1). Killing older embryos before hatching required doses of several thousand R, but the effective lethal phase for the damage induced before an age of 7 hours was not reached until hatching, which normally occurred at 22 hours. Once the differentiation of the larval organs was completed, embryonic development and larval life seemed no longer affected at doses below the LD_{100} emergence range. Instead, in these stages differentiation and development of the imaginal discs was affected. Again, distinct sensitive and effective phases were found.

The sensitive phases were interpreted as stages of high gene action [9]. This point of view was confirmed by radiation-induced phenocopies – modifications copying the phenotypes of mutants. They were inducible at sensitive phases thought to represent those developmental stages at which the genes in question normally become active. For example, the mutant Dichaete of D. melanogaster had dorsocentral bristles reduced in number and the wings extended to 45° from the body axis. Fritz-Niggli [20] saw the same malformations in up to 100% of the surviving flies after irradiation of 5-hour old prepupae. They were not found after exposure of larvae or of older pupae. The question arises whether one of the primary radiation effects is some kind of somatic

mutation. These were investigated by Patterson [23] in 1929, and recently in several genetic [24] and cytogenetic [25] studies. The idea of their lethal action on developmental stages was strengthened by some findings in Drosophila. According to Ulrich [26], in the young embryo the nucleated part of the egg is 182 times as sensitive as the cytoplasm. Würgler [10] found that during cleavage the relatively lowest sensitivity appeared in the interphase stages (Fig. 1). Several authors showed by means of special genetic stocks that the lethal radiation effects induced in larvae were: "almost exclusively due to chromosome breakage followed by chromosome loss, cellular damage or death resulting whenever a portion of the genome was no longer represented in at least haploid condition" (Ostertag [27]).

Hence, in the developmental stages of insects, somatic mutations, radiation-induced predominantly during mitotic divisions, result in death until emergence or in modifications visible in the adults. One rather unspecific group of such modifications is the shortening of adult lifespan (Table II), which depends on the developmental stage exposed and on the dose. Since in the adult insect differentiation is completed and mitotic activity has ceased in the somatic tissues, a quite different radiation response should be expected when the 'aging' adult insect is exposed.

TABLE II. EFFECTS OF X-IRRADIATING DEVELOPMENTAL STAGES OF HABROBRACON JUGLANDIS WITH DIFFERENT DOSES ON MEAN ADULT LIFESPAN (M). - Data from CLARK (21) and ERDMAN (22)

Embryos (males)		Larvae (females)		white Pupae (females)	
dose in R	M	dose in R	M	dose in R	M
600	23 days	0	28 days	0	29 days
900	16 days	1,000	24 days	5,000	22 days
1,200	14 days	2,000	11 days	10,000	12 days
1,500	8 days	3,000	6 days	15,000	10 days

3. RADIATION EFFECTS ON YOUNG ADULTS

The induced reduction of mean lifespan after irradiation of adult D. melanogaster males and females with various doses is shown in Fig. 2. The dose-action relationships indicate a much lower radiosensitivity of adults compared with that of the developmental stages. These relationships were linear, but consisted of two parts, separated by a steepening. Each part was believed to indicate at least one type of mortality. The early death, found at high doses, is called 'type 1 mortality' in this paper. The more delayed death is called 'type 2 mortality'. The dose-action part of the latter was interrupted in females by a considerable prolongation of lifespan.

3.1. Type 1 mortality

A change in the type of mortality at high doses was confirmed (Fig. 3). The accumulated mortality rates after irradiation of Drosophila females with different doses are here given on a probability scale. A Gauss

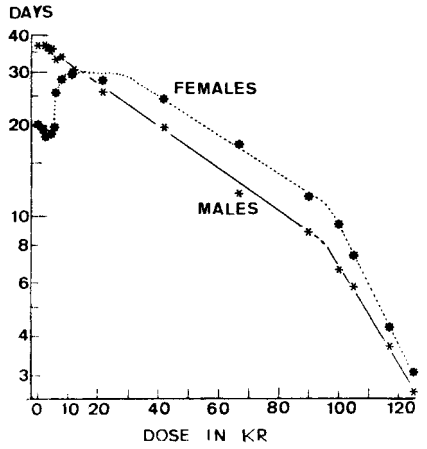

FIG. 2. Mean survival times in days after X-irradiation of D. melanogaster males and females with different doses. After Nöthel (28).

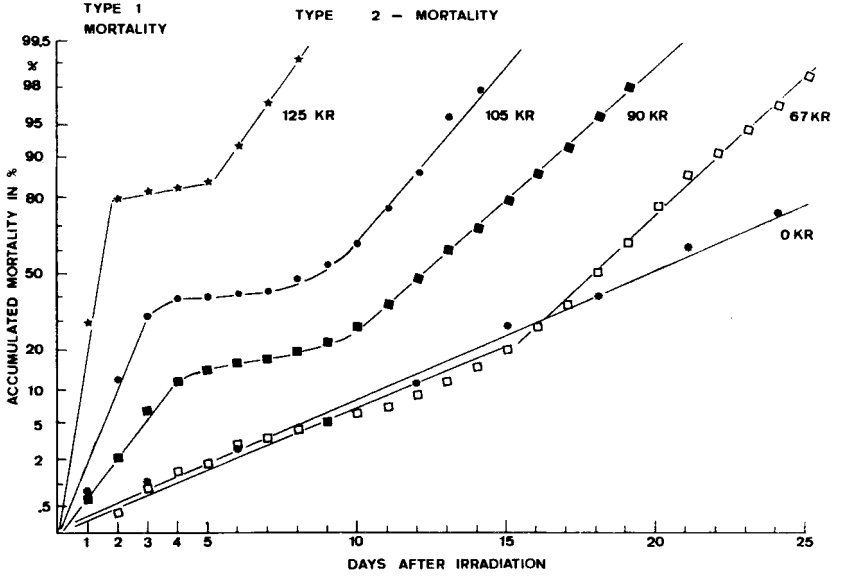

FIG. 3. Accumulated mortality rates as a function of time after X-irradiation of D. melanogaster females with high doses. Data after Nöthel (28).

distribution of percentage accumulated mortality resulted in a linear regression line. This linearity was apparent in the unirradiated controls (0 kR). It persisted at 67 kR, but had an increased regression coefficient after an initial latent period. However, beginning at 90 kR the distribution became more and more bimodal, an early type of induced death overlapping the 'normal' one.

Flies showing type 1 mortality were characterized by a special radiation syndrome: they were very quiet, unable to fly one day after

exposure, and most of them showed unco-ordinated movements of locomotion organs. These characteristics in Drosophila were similar to those of a coma induced at high doses in Habrobracon [29] and Dahlbominus [30]. It was concluded that type 1 mortality was due to damage in the central nervous system. This is considered common in all insects irradiated with high doses, since at a dose level of 150 to 200 kR, the T_{50} did not exceed a few days in all insects tested (Table III). The T_{50} is the time at which 50% of the animals investigated died. By means of this T_{50}, the radiation responses of various species are compared at different dose levels in Table III.

TABLE III. T_{50} IN DAYS AFTER IRRADIATION OF NEWLY EMERGED ADULTS OF VARIOUS INSECT SPECIES AT DIFFERENT DOSE LEVELS (IN kR)

Insect	dose in kR:	0	10-20	50-70	150-200	References
ORTHOPTERA						
Periplaneta americana	(males)	200	10	4	-	WHARTON (31)
COLEOPTERA						
Tribolium confusum		290	20	-	-	CORK (32)
		-	12	9	3	HASSETT (33)
Rhyzoperta dominica		100	23	14	3	HASSETT (33)
Epilachna varifestis	(males)	34	16	-	-	HENNEBERRY(34)
Pissobes strobi	(males)	34	16	-	-	JAYNES (35)
Anthonomus grandis		30	10	-	-	DAVICH (36)
HYMENOPTERA						
Habrobracon juglandis	(females)	25	13	12	6	CLARK (21)
Habrobracon serinopae	(males)	62	36	18	-	CLARK (37)
DIPTERA						
Aedes aegypti	(females)	45	35	-	-	STAHLER (38)
Drosophila subobscura	(males)	47	34	14	-	LAMB (39)
Drosophila melanogaster	(males)	44	38	13	5	BAXTER (40)
		37	26	11	2	NÖTHEL (28)

3.2. Type 2 mortality

According to Table III, within the dose range of type 2 mortality, differences between the various insects are apparent, especially at low doses (10-20 kR). These differences become clearer by consideration of two examples: (a) Dose-action curves in Drosophila and Periplaneta males are compared in Fig. 4. In contrast to the findings in Drosophila, in Periplaneta the decrease of T_{50} with increasing dose was bimodal even at low doses; it was considerable between 2.5 and 10 krad and levelled off at higher doses. (b) Accumulated mortality rates (on a probability scale) of irradiated and control males of Drosophila and Tribolium are compared in Fig. 5. In the controls the regression lines are straight in both species. After irradiation, this type of regression persisted in Drosophila, but in Tribolium the post-irradiation mortality distribution was bimodal. A sharp initial increase was followed by a return to the control rates. As to other doses, in Tribolium the initial increase goes up to 45% accumulated mortality at 11 kR and to 100% at 20 kR. In

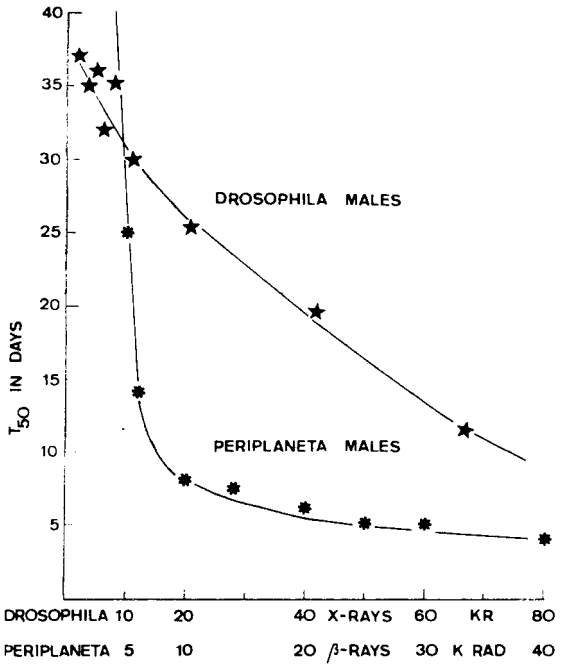

FIG. 4. T_{50} as a function of dose after irradiation of Drosophila and Periplaneta males. Periplaneta data after Wharton (31), Drosophila data after Nöthel (28).

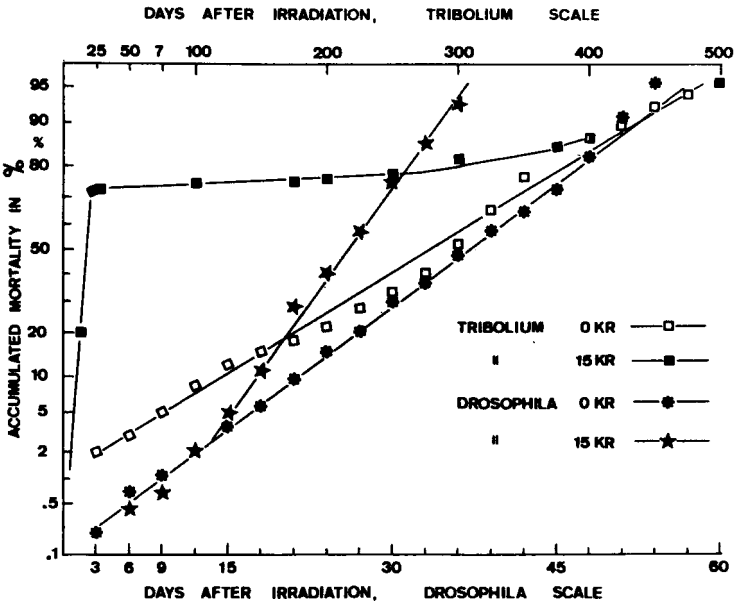

FIG. 5. Accumulated mortality rates as a function of time in Drosophila and Tribolium males with and without irradiation. Tribolium data after Cork (32), Drosophila data after Nöthel (28).

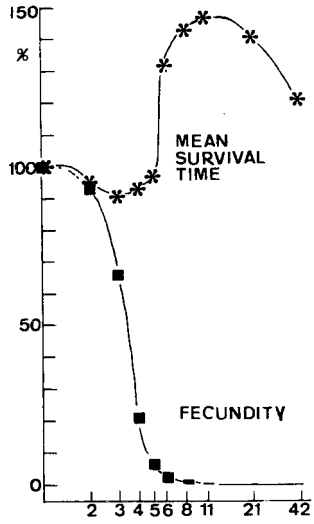

FIG. 6. Fecundity (after exposition of oögonia) and mean survival times of D. melanogaster females after X-irradiation with different doses in per cent of unirradiated controls (0 kR). After Nöthel (28).

Drosophila a corresponding distribution was not found at low or median doses. Hence it was assumed that different injuries occurred within type 2 mortality in the various insect species.

In Drosophila there existed some heterogeneity within type 2 mortality, as became apparent in two examples associated with radiation-induced sterilization of females: (a) A prolongation of female lifespan (Fig. 2) is shown in Fig. 6 to be correlated with sterilization. According to Nöthel [28, 41, 42], it was due to the sterilization, since sterilization with TEM resulted in the same prolongation effect as irradiation with 11 kR. Lamb [39] found a corresponding prolongation in normal females, but not in those which genetically lack the ovaries. (b) An opposite effect of female sterilization on lifespan was found to be due to a special hereditary factor (Nöthel, unpublished). Its action is shown in Table IV. Normal females were permanently sterilized by dominant lethals induced in the oögonia. Killing one oögonial cell resulted in the resorption of one ovariole [43]. 100% sterilization was induced at 11 kR [5], at which dose the ovarioles diminished some time after exposure. In the carriers of the factor in question, permanent sterilization was induced at 4 kR, but in these females oögenesis was not affected and no ovarioles were resorbed. Instead, oviposition was blocked, the ovaries became enlarged and the fly died very soon because it could not get rid of the egg masses.

Several attempts were made with various insects to understand the nature of type 2 mortality. Experiments with split doses, starvation, nitrogen or phosphorus turnover, or other metabolic parameters did not yield sufficient information. Studies with different ploidy levels favoured the view that even in adult insects somatic mutations were a primary effect of radiation injury. Clark [21, 37] used haploids and diploids of Habrobracon. He found that the radiation-induced decrease in lifespan was markedly influenced by genome number (Fig. 7) and concluded: "that

TABLE IV. THE BEARING ON T_{50} OF A SPECIAL GENE-CONTROLLED TYPE OF RADIATION-INDUCED STERILITY IN D. MELANOGASTER FEMALES

	eggs/female		T_{50} in
	days 1 + 2	days 8-11	days
normal females:			
0 kR		174	28
4 kR	122	154	27
females with the factor for "induced sterility":			
0 kR		174	28
4 kR	129	0	11

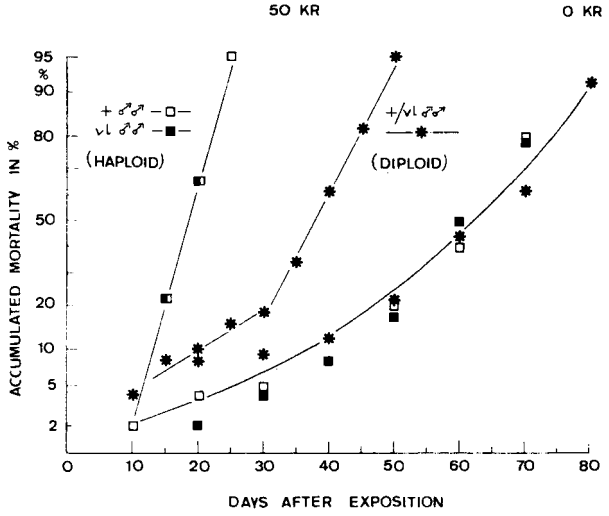

FIG. 7. Accumulated mortality rates as a function of time in haploid (+ or vl) and diploid (+/vl) males of Habrobracon serinopae with and without X-irradiation. After Clark and Rubin (37).

the decrease in lifespan by X-irradiation is due to damage of a genetic nature" [37]. Clark found considerable differences between radiation-induced mortality and natural death, since, according to the latter, haploids and diploids behaved similarly (Fig. 7). On the other hand, at least in Drosophila, the shape of the dose-action curves as well as that of the accumulated mortality rates favoured the idea of 'radiation-induced aging' with regard to type 2 mortality.

4. RADIATION EFFECTS ON AGING ADULTS

With increasing age at irradiation the adult insect died with decreasing doses [31]. Some recent papers of Baxter and Blair [40], Lamb [44], and Nöthel [45] dealt with this phenomenon in Drosophila. Since there were several discrepancies in the interpretations given by the authors cited, we performed some additional experiments with D. melanogaster. The results are described here, but the material and methods used, as well as detailed statistics, will be published elsewhere.

4.1. Type 1 mortality

In our previous investigations [45], the LD_{50} type 1 mortality decreased rather rapidly with increasing age at irradiation (Fig. 8). The decrease was similar in both sexes, whereas natural aging depended on sex, as indicated by mean lifetimes of 41.8 and 29.9 days in males and females, respectively. Hence it was concluded that the increase in radiation sensitivity was due to aging of the central nervous system (responsible for type 1 mortality but not for natural death) rather than to that of the whole organism [45]. The experimental data of Baxter and Blair [40] were in good agreement with our findings, except for one rather important point: these authors found the rates of aging as indicated by the radiation response to be inversely proportional to normal lifespans of males and females.

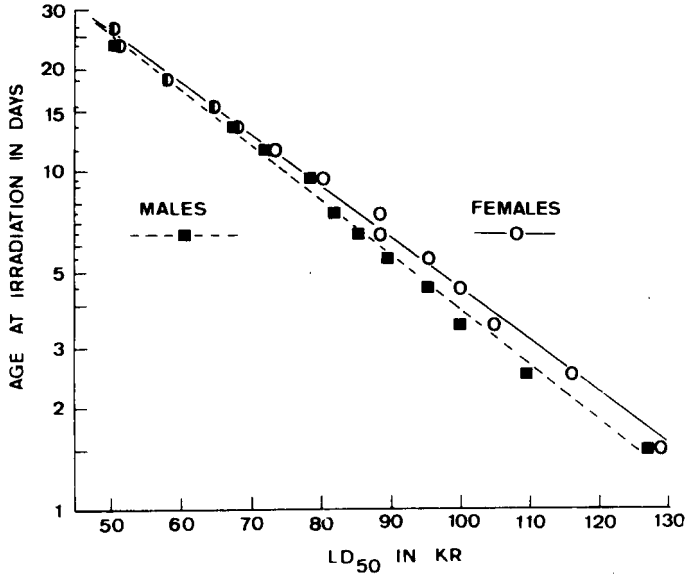

FIG. 8. LD_{50}-type 1 mortality as a function of age at X-irradiation in D. melanogaster males and females. After Nöthel (45).

Both interpretations were tested with the age dependence of type 1 mortality in males and females of four additional and different D. melanogaster stocks. These exhibited considerably different lifespans

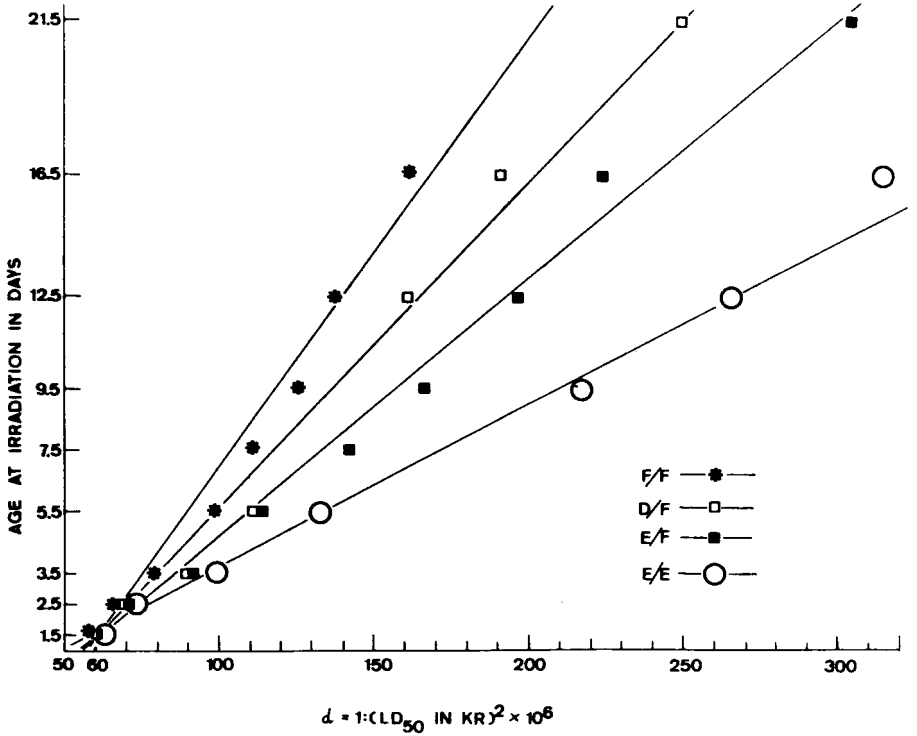

FIG. 9. LD_{50}-type 1 mortality as a function of age at X-irradiation in D. melanogaster males of different strains. Mean lifespans of unirradiated males in days:
E/E 25.9±.8, F/F 18.3±.9, D/F 43.4±1.3, E/F 31.8±1.3.

when unirradiated (Fig. 9) and were therefore believed to have different rates of natural aging. To establish practicable age-to-LD_{50} relationships, the reciprocals of the squares of LD_{50} values were used; $10^6 : (LD_{50}$ in kR$)^2$ was symbolized by d. In Fig. 9, d proves to be a linear function of age at exposure, distinct in every genotype and sex. Natural aging was measured by the accumulated mortality rates, given in probit units Z. Z was a linear function of age in every genotype and sex (see Figs 4 and 12, for example). Since the age is a linear function of d as well as of Z, Z is given in Fig. 10 as a function of d for every genotype and sex tested. If, indeed, the radiation susceptibility (indicated by d) depended on aging as an overall effect (indicated by Z), the function $Z = f(d)$ should be equal in every genotype and sex. According to Fig. 10, this holds true only in some cases. Therefore, both natural aging as indicated by lifespan and aging of the central nervous system as indicated by radiation-induced type 1 mortality were fixed by genotype, as was the mode of correlation between them.

4.2. Type 2 mortality

In Drosophila, type 2 mortality looked like 'radiation-induced aging'. Lamb and Maynard Smith [46] found the percentage reduction in lifespan

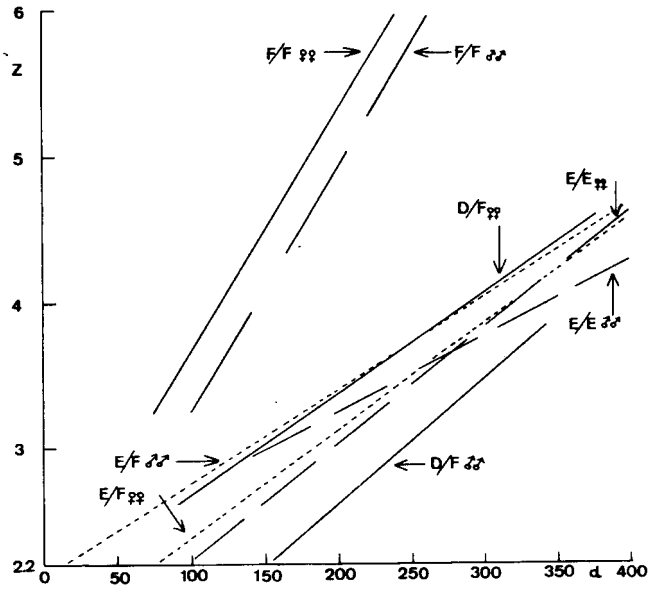

FIG. 10. Accumulated natural mortality rates (Z) as a function of radiation sensitivity to type 1 mortality (d) in D. melanogaster males and females of different strains.

Z = probit units of percentage accumulated mortality
= linear function of age in unirradiated controls.
$d = 10^6 : (LD_{50}\text{-type 1 mortality in kR})^2$
= linear function of age at irradiation.

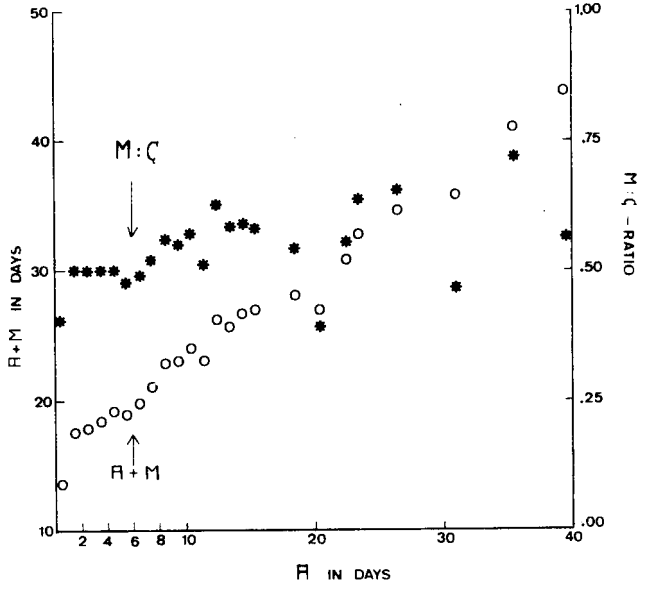

FIG. 11. Relations in D. melanogaster males between the age (A) at X-irradiation with 42 kR, mean postirradiation survival time (M), and mean control survival time (C) at the same age.

by a given dose to be always the same whatever the control lifespan (varied by temperature) was. Lamb [44] found the same constant relation after irradiation at different ages. It was, however, not clear whether type 2 mortality depended on the aging processes involved in the shortening of natural life expectancy or whether it reflected alterations of some other metabolic properties with age. To test this, we paid special attention to the early days of adult life, since during this period of 'metachemogenesis' there is an increase in various physiological properties and a decrease in natural life expectancy [47].

According to Fig. 11, the mean survival times after irradiation at different ages were in a nearly constant relation to those of controls at the same age. The sum of pre- plus post-irradiation lifetimes proved to be far from constancy. These results are in agreement with those of Lamb [44] and contrary to those of Baxter and Blair [40].

However, in using the mean survival times only, some simplifications were made. It seemed more appropriate with our experimental material to use the accumulated mortality rates. Some examples of this form of consideration are given in Fig. 12. The backbone is the regression line of unirradiated controls. The lines of the irradiated samples start from it, and to obtain them the sum of pre-and post-irradiation deaths was set at 100%. The post-irradiation mortality curves consisted of two distinct linear components. The first was equal to the natural mortality and represented a latent period. The second exhibited a much stronger increase and represented the 'induced mortality'. Latent period and median survival time within the induced mortality were evaluated for every age class by means of probit equations. They are compared in Fig. 13. It is evident that, independently of age at irradiation, the same time is always required until 50% of the animals have died within the induced mortality period. The latency, however, increases with increasing age at exposure up to a maximum at about the third day, and then decreases. The initial increase in latency was similar to the metachemogenesis of some enzyme activities in Musca [47] and to the increase in respiration rate [48] or oviposition rate [5] in Drosophila. Hence it is probable that the age-dependent alterations in the sensitivity to type 2 mortality were due to specific metabolic changes rather than to natural aging itself, and that this mortality was not an 'induced aging'.

5. SUMMARY AND CONCLUSIONS

The non-genetic overall radiation response of insects is expressed by the fitness components 'developmental rate' and 'adult lifespan' and is hence related to natural aging. In the developmental stages, ionizing radiation affects the differentiation processes, at least in part, by means of somatic mutations. With increasing differentiation, the radiation injury decreases until a maximum resistance is achieved about emergence. In the adults, the sensitivity again increases with increasing age at exposure. However, this correlation does not reflect true interactions between natural aging and radiation effects, such as 'induced accumulated aging'. Differences between both parameters indicate that the sensitization is a secondary effect of metabolic alterations. Therefore, the radiation

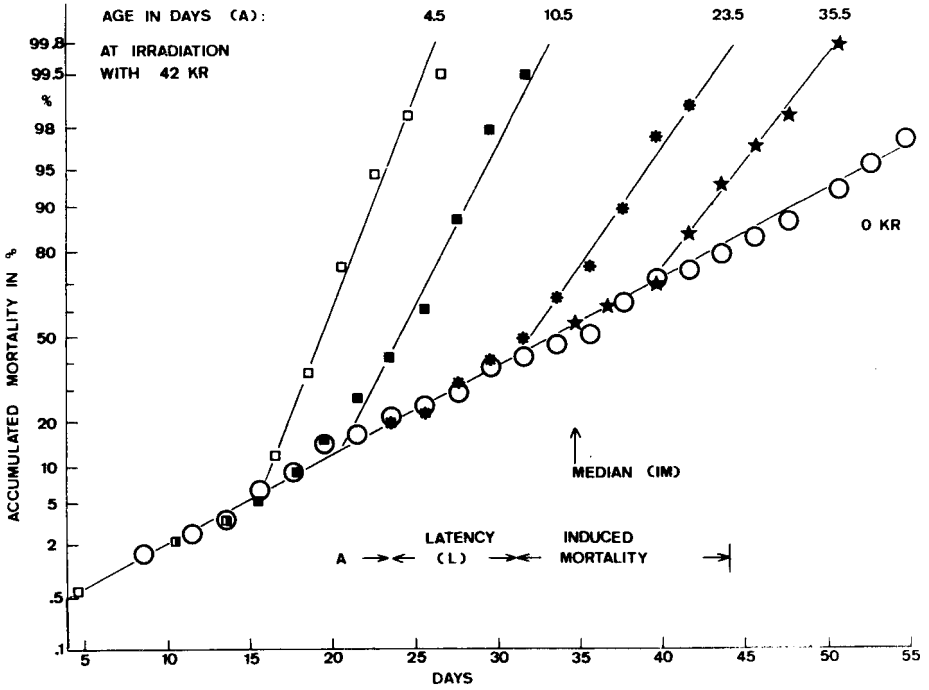

FIG. 12. Accumulated mortality rates after X-irradiation of D. melanogaster males at various ages with 42 kR.

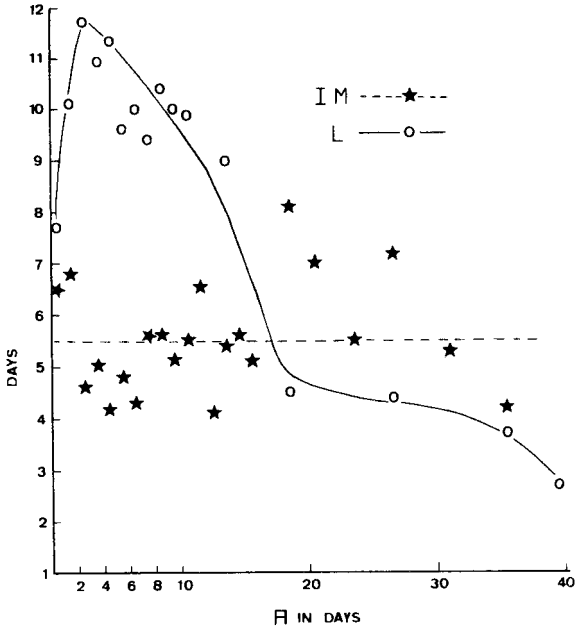

FIG. 13. Latent periods (L) and median survival times within the 'induced mortality' periods (IM) in days after X-irradiation of D. melanogaster males at various ages with 42 kR.

response of insects is not such a good tool to establish a model of natural aging as has often been predicted.

The radiobiological investigations of developmental stages turned out to be studies in developmental genetics. In the adults, the interspecific variations in the radiation response will not be explained until there is additional knowledge on comparative insect physiology. The intraspecific variability, however, promises good results. This is seen in studies with different ploidy levels in Habrobracon or even in those on secondary effects on longevity by sterilizing Drosophila females. Hence the study of non-genetic radiation effects by means of well known genetic deviants should be propagated.

REFERENCES

(1) DEMEREC, M., FANO, U., Frequency of dominant lethals induced by radiation in sperms of Drosophila melanogaster. Genetics 29 (1944) 348-360.

(2) Von BORSTEL, R.C., REKEMEYER, M.L., Radiation-induced and genetically contrived dominant lethality in Habrobracon and Drosophila. Genetics 44 (1959) 1053-74.

(3) LACHANCE, L.E., RIEMANN, J.G., Cytogenetic investigations on radiation and chemically induced dominant lethal mutations in oocytes and sperm of the screw-worm fly. Mutation Res. 1 (1964) 318-333.

(4) ERDMAN, H.E., Modifications of productivity in flour beetles, Tribolium castaneum Herbst, due to X-ray doses, hypothermia, and the sex exposed. Radiat. Res. 25 (1965) 341-351.

(5) NÖTHEL, H., Der Einfluß von Röntgenstrahlen auf Vitalitätsmerkmale von Drosophila melanogaster. II. Untersuchungen über die Fekundität. Strahlentherapie 134 (1967) in press.

(6) NÖTHEL, H., --- IV. Untersuchungen über die Fertilität. Strahlentherapie, in press.

(7) FRITZ-NIGGLI, H., Biologische Analyse der Strahlenschädigung von Drosophila-Eiern durch 180 kV Röntgenstrahlen und ultraharte 31 MeV-Strahlen. Naturwissenschaften 39 (1952) 485-486.

(8) FRITZ-NIGGLI, H., Vergleichende Analyse der Strahlenschädigung von Drosophila-Eiern mit 180 keV und 31 MeV. Fortschr. Röntgenstr. 83 (1955) 178-200.

(9) FRITZ-NIGGLI, H., "Strahlenbiologie", Thieme, Stuttgart (1959).

(10) WÜRGLER, F.E., ULRICH, H., SCHNEIDER-MINDER, A., Variation of radiasensitivity during meiosis and early cleavage in newly laid eggs of Drosophila melanogaster. "Repair from Genetic Radiation Damage and Differential Radiasensitivity in Germ Cells (SOBELS, F.H. Ed.), Pergamon Press, Oxford (1963) 101-106.

(11) KHISHIN, A.F.E., The response of the immature testis of Drosophila to the mutagenic action of X-rays. Z. Vererbungslehre 87 (1955) 97-112.

(12) AUERBACH, Ch., Sensitivity of the Drosophila testis to the mutagenic action of X-rays. Z. Vererbungslehre 86 (1954) 113-125.

(13) MAVOR, J.W., A comparison of the susceptibility to X-rays of Drosophila melanogaster at various stages of its life-cycle. J. exp. Zool. 47 (1927) 63-83.

(14) AMY, R.L., A comparative study of the effects of ß-rays, γ-rays, and X-rays on development in Habrobracon. Radiat. Res. 3 (1955) 166-181.

(15) CLARK, A.M., The relation of genome number to radiosensitivity in Habrobracon. Amer. Naturalist 91 (1957) 111-119.

(16) ERDMAN, H.E., Analyses of the differential radiosensitivity of developing reproductive tissues in Habrobracon juglandis (Ashmead) to ionizing radiation. Int. J. Rad. Biol. 3 (1961) 183-204.

(17) BALOCK, J.W., BURDITT, A.K. jr., CHRISTENSON, L.D., Effects of gamma radiation on various stages of three fruit fly species. J. Econ. Entomol. 56 (1963) 42-46.

(18) BENSCHOTER, C.A., TELICH, J., Effect of gamma rays on immature stages of the mexican fruit fly. J. Econ. Entomol. 57 (1964) 690-691.

(19) STEENBECK, L., Histologische Untersuchungen zum "Frühtod" röntgenbestrahlter Drosophila-Eier. Naturwissenschaften 48 (1961) 310.

(20) FRITZ-NIGGLI, H., Erste biologische Versuche mit dem 31-MeV-Betatron. Arch. Klaus-Stift. Vererb. Forsch. 24 (1951) 454-459.

(21) CLARK, A.M., Some effects of X-irradiation on longevity in Habrobracon females. Radiat. Res. 15 (1961) 515-519.
(22) ERDMAN, H.E., Adult longevity as a sensitive criterion of radiation-induced damage when 24-hour Habrobracon (Hymenoptera) embryos are X-rayed. J. Econ. Entomol. 53 (1960) 971-972.
(23) PATTERSON, J.T., The production of mutations in somatic cells of Drosophila melanogaster by means of X-rays. J. exp. Zool. 53 (1929) 327-372.
(24) TOKUNAGA, Ch., ARNHEIM, N. jr., Age dependence of the locations of X-ray induced somatic crossing over in Drosophila. Genetics 54 (1966) 267-276.
(25) FOX, D.P., The effect of X-rays on the chromosomes of locust embryos. I. The early responses. Chromosoma (Berl.) 19 (1966) 300-316.
(26) ULRICH, H., Ein Vergleich der Röntgenstrahlenwirkung auf Kern und Plasma des Drosophila-Eies. Biol. Zentralblatt 74 (1955) 498-515.
(27) OSTERTAG, W., The genetic basis of somatic damage produced by radiation in third instar larvae of Drosophila melanogaster. Z. Vererbungslehre 94 (1963) 143-162.
(28) NÖTHEL, H., Der Einfluß von Röntgenstrahlen auf Vitalitätsmerkmale von Drosophila melanogaster. I. Untersuchungen über die Lebensdauer. Strahlentherapie 126 (1965) 269-282.
(29) HEIDENTHAL, G., The occurence of X-ray induced dominant lethal mutations in Habrobracon. Genetics 30 (1945) 197-205.
(30) BALDWIN, W.F., Similarities in killing by heat and by X-irradiation in the insect Dahlbominus fuscipennis (Zett.). Radiat. Res. 5 (1956) 46-51.
(31) WHARTON, D.R.A., WHARTON, M.L., The effect of radiation on the longevity of the cockroach, Periplaneta americana, as affected by dose, age, sex and food intake. Radiat. Res. 11 (1959) 600-615.
(32) CORK, J.M., Gamma-radiation and longevity of the flour beetle. Radiat. Res. 7 (1957) 551-557.
(33) HASSETT, C.C., JENKINS, D.W., Use of fission products for insect control. Nucleonics 10 (1952) 12 42-46.
(34) HENNEBERRY, T.J., SMITH, F.F., McGOVERN, W.L., Some effects of gamma radiation and a chemosterilant on the mexican bean beetle. J. Econ. Entomol. 57 (1964) 813-815.
(35) JAYNES, H.A., GODWIN, P.A., Sterilization of the white-pine weevil with gamma radiation. J. Econ. Entomol. 50 (1957) 393-395.
(36) DAVICH, T.B., LINDQUIST, D.A., Exploratory studies on gamma radiation for the sterilization of the boll weevil. J. Econ. Entomol. 55 (1962) 164-167.
(37) CLARK, A.M., RUBIN, M.A., The modification by X-irradiation of the life span of haploids and diploids of the wasp, Habrobracon sp. Radiat. Res. 15 (1961) 244-253.
(38) STAHLER, N., TERZIAN, L.A., The response of blood-fed Aedes aegypti to gamma radiation. J. Econ. Entomol. 56 (1963) 416-417.
(39) LAMB, M.J., The effects of radiation on the longevity of female Drosophila subobscura. J. Ins. Physiol. 10 (1964) 487-497.
(40) BAXTER, R.C., BLAIR, H.A., Kinetics of aging as revealed by X-ray-dose-lethality relations in Drosophila. Radiat. Res. 30 (1967) 48-70.
(41) NÖTHEL, H., The influence of X-rays on longevity, fecundity and fertility of Drosophila melanogaster. Int. J. Rad. Biol. 6 (1963) 381-382.
(42) NÖTHEL, H., Different types of mortality including prolongation of female lifetime after X-raying Drosophila melanogaster imagines. "Genetics Today", Proc. XI Int. Congr. Genetics, The Hague, 1 (1963) 72-73.
(43) NÖTHEL, H., An estimation of the number of active oogonia per ovariole in D. melanogaster females. DIS 42 (1967) 55.
(44) LAMB, M.J., The relationship between age at irradiation and life-shortening in adult Drosophila. "Radiation & Ageing" (LINDOP, P.J., SACHER, G.A., Eds.), Taylor & Francis, London (1966) 163-174.
(45) NÖTHEL, H., Der Einfluß von Röntgenstrahlen auf Vitalitätsmerkmale von Drosophila melanogaster. III. Untersuchungen über den Einfluß des Alters auf die Strahlenempfindlichkeit. Strahlentherapie 135 (1968) in press.
(46) LAMB, M.J., MAYNARD SMITH, J., Radiation and ageing in insects. Exp. Gerontol. 1 (1964) 11-20.
(47) CLARK, A.M., ROCKSTEIN, M., Aging in insects. "The Physiology of Insecta" (ROCKSTEIN, M., Ed.), Academic press, New York, 1 (1964) 227-281.
(48) KERR, R.W., Variation with age in the susceptibility to DDT and the respiration rate of male and female Drosophila melanogaster Mg. Bull. ent. Res. 45 (1954) 323-328.

DESTRUCTION DES CELLULES POLAIRES DE L'ŒUF DU DORYPHORE PAR IRRADIATION

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Abstract — Résumé

DESTRUCTION DES CELLULES POLAIRES DE L'ŒUF DU DORYPHORE PAR IRRADIATION. L'irradiation localisée, au moyen de rayons X de faible énergie, du pôle postérieur de la blastula de Leptinotarsa decemlineata Say (Chrysomelidæ) est réalisée sans entraver la poursuite du développement embryonnaire. L'examen histologique des gonades des larves de premier stade montre qu'elles sont totalement dépourvues de gonies lorsque la dose est suffisamment élevée (10 000 R) pour entraîner la destruction certaine des cellules polaires postérieures. L'existence de gonades agamétiques, différenciées à l'image des témoins, montre que l'édification de la partie mésodermique de la gonade est indépendante de la présence des cellules germinales initiales. Les larves agamétiques ne sont pas viables, sauf exception, et deux hypothèses sont émises pour tenter d'expliquer ce fait. Il semble qu'un phénomène de régulation intervienne au cours du développement larvaire ou nymphal des gonades dont le nombre de gonies est inférieur à celui des témoins (cas des œufs irradiés à 3000 R).

DESTRUCTION OF THE POLAR CELLS OF COLORADO BEETLE EGGS BY IRRADIATION. The rear pole of the blastoderm of Leptinotarsa decemlineata Say (Chrysomelidae) is subjected to local irradiation with low-energy X-rays without disruption of embryonic development. Histological study of the gonads of growing larvae shows total absence of gonia after a sufficiently high dose (10 000 R) to cause certain destruction of the rear polar cells. The existence of agamic gonads differing in appearance from those in the control cells indicates that development of the mesodermic part of the gonad is independent of the presence of initial germinal cells. Two hypotheses are put forward to explain the fact that agamic larvae are almost always non-viable. It would appear that in cases where the number of gonia is less than in the control cells (e.g. eggs irradiated at 3000 R), a regulation phenomenon has come into play in the course of gonial development in the larva or pupa.

1. ETUDE BIBLIOGRAPHIQUE ET BUT DU TRAVAIL

Entre 1908 et 1911, Hegner [1, 2] mettait en évidence l'existence de deux tissus, bien distincts par leur origine, dans la gonade du doryphore (Leptinotarsa decemlineata Say, Chrysomélide, Coléoptère). Au moyen d'une aiguille chaude, il détruisait le pôle postérieur de l'œuf, au stade blastula, et constatait l'absence de cellules germinales dans les gonades des embryons issus des œufs opérés. Geigy [3], puis Aboim [4], réalisèrent le même type d'expérience chez Drosophila au moyen d'une brûlure par rayonnement UV et confirmèrent l'existence de gonades agamétiques; ils montrèrent que les gonades de ce diptère sont constituées de deux masses mésodermiques où viennent se loger, après migration, les cellules polaires, initiales des cellules germinales. Selon ces auteurs, l'édification de ces deux masses mésodermiques est indépendante de la présence des cellules germinales. Depuis, différents auteurs ont obtenu des résultats similaires: Haget [5] chez Leptinotarsa,

Alleaume et Haget [6] chez Calliphora par microcautérisation, Hataway et Selman [7] chez Drosophila, Oelhafen [8] chez Culex, Jura [9] chez Drosophila par irradiation UV localisée, Idris [10] chez Culex par ligature.

A l'opposé, Counce et Selman [11], soumettant des œufs de Drosophila à un traitement par les ultra-sons, ont obtenu deux embryons ayant des gonades situées dans la région antérieure, à proximité de l'œsophage: pour ces auteurs, les cellules polaires ont atteint une masse mésodermique antérieure et induit à cet endroit la formation des gonades. Les observations de Counce et Ede [12] sur un mutant de Drosophila, et les résultats de Poulson et Waterhouse [13] viennent à l'appui de cette hypothèse. Ces derniers auteurs, après brûlure des cellules polaires de l'œuf de Drosophila par rayonnement UV, constatèrent, soit la présence de quelques gonies dans les gonades des embryons obtenus, soit l'absence totale de gonades. Ils conclurent que la présence de cellules polaires est nécessaire à l'édification de la partie mésodermique de la gonade. Anderson [14], s'appuyant sur les trois travaux cités ci-dessus et sur celui de Davis (non publié) chez Culex et Lucilia, conclut à l'existence indiscutable de l'induction de la partie mésodermique de la gonade par les cellules polaires. Il considère en effet que les gonades agamétiques, mises en évidence par de nombreux auteurs, ne sont pas intégralement mésodermiques.

Ce travail a eu pour but de fournir des indications sur le rôle des cellules polaires, en les détruisant au moyen d'une nouvelle technique: l'irradiation localisée du pôle postérieur de la blastula par des rayons X de faible énergie. Cette étude a été poursuivie principalement dans deux directions:

- existence et structure des gonades,
- possibilités de développement après l'irradiation.

2. MATERIEL ET TECHNIQUES

Les œufs, collés par la femelle sur une feuille de pomme de terre, sont récoltés aussitôt pondus. Ils sont placés à 16°C dans une enceinte humide. Dans ces conditions de développement, le stade blastula jeune (1000 à 2000 noyaux) est atteint en 24 h, le stade blastula âgé (16 000 noyaux) en 48 h [15]. Ils sont placés sur une plaque de plomb de 5 mm d'épaisseur, enduite d'une mince couche de graisse à vide. Cette plaque est percée de canaux ayant 0,5 mm de diamètre. Les œufs sont disposés de façon telle que seule la partie polaire postérieure (1/8 de la longueur totale) soit atteinte par les rayons X lorsque la plaque, retournée, est placée sur une enceinte en plomb.

Les irradiations sont effectuées au moyen d'un tube Holweck muni d'une anticathode en molybdène et d'une fenêtre en aluminium (0,05 mm), émettant un rayonnement X peu pénétrant: la couche de demi-absorption est égale à 1 mm d'eau. L'appareil fonctionne sous une tension de 37,5 kV et avec une intensité de 15 mA. La dosimétrie, effectuée d'une part par la méthode chimique au sulfate ferreux et d'autre part avec un dosimètre Phillips, indique un débit de 3 000 R/min à 118 mm de la fenêtre. Les doses utilisées, 1 000 R à 30 000 R, sont délivrées à débit constant, les durées d'irradiation variant de 20 s à 10 min.

Après irradiation les œufs sont incubés à 25°C dans une enceinte humide. Les larves sont tuées à la naissance au moyen de vapeurs de chloroforme puis fixées par le mélange de Carnoy durant 1 h (acide acétique: 1 volume; chloroforme: 3 volumes; alcool absolu: 6 volumes). Les larves sont ensuite incluses dans la paraffine (54-56°C) après déshydratation par l'alcool butylique. Les coupes sériées (6 μm) sont colorées par un mélange d'érythrosine-aurantia-orangé G (5 min) puis par le bleu de toluidine (1 min 30 s), déshydratées par le benzène et montées dans le beaume du Canada.

3. ETUDE DES GONADES

Témoins

Les gonades de la larve naissante du doryphore se présentent sous deux aspects selon le sexe: deux masses ovoïdes chez la femelle, deux haltères chez le mâle. Elles sont situées dans la partie postérieure de l'abdomen, de chaque côté de l'intestin moyen. Elles sont entourées d'une gaine de grosses cellules adipeuses. A l'intérieur de chaque gonade on distingue deux catégories cellulaires:
- de petites cellules banales, d'origine mésodermique,
- de grosses cellules caractérisées par un noyau sphérique, très volumineux (diamètre > 10 μm), faiblement colorable et contenant un gros nucléole; une mince couche de cytoplasme périphérique très basophile (coloration intense par le bleu de toluidine).

Ces cellules, gonies primordiales, sont plus nombreuses dans la gonade mâle, où elles sont réparties de façon égale dans les deux renflements.

Larves issues d'œufs irradiés

Irradiation au stade blastula jeune

1000 R (22 cas): 40% de gonades dépourvues de gonies
40% de gonades ayant un nombre variable de gonies
20% de gonades normales.

3000 R (28 cas): 70% de gonades dépourvues de gonies
30% de gonades ayant un nombre variable de gonies.

10 000 R (36 cas) et 30 000 R (20 cas): toutes les gonades sont dépourvues de gonies, à l'exception de trois cas, probablement dus à une mise en place des œufs défectueuse au moment de l'irradiation.

Irradiation au stade blastula âgée

10 000 R (24 cas): 60% de gonades dépourvues de gonies
40% de gonades ayant un nombre variable de gonies.

Aspect des gonades

Dans tous les cas examinés, deux gonades bien distinctes existent. Elles sont toujours situées à leur emplacement anatomique normal, mais, le plus souvent, les gonies primordiales manquent totalement ou sont peu nombreuses. Les gonades, dépourvues de gonies, ont une taille nettement inférieure à celle des gonades témoins, mais conservent parfaitement leur forme: ovoïde ou en haltère.

Discussion

Ces expériences montrent clairement que la partie mésodermique de la gonade peut s'édifier et se différencier selon le sexe, malgré l'absence des cellules germinales initiales. Cette conclusion va à l'encontre de celle de certains auteurs [13, 14] qui, après destruction des cellules polaires, n'ont pas constaté la présence de gonades agamétiques. Il est raisonnable de penser que ces auteurs ont peut-être détruit, outre les cellules polaires, les cellules blastodermiques qui fournissent normalement le mésoderme de la région moyenne abdominale [5, 6].

4. POSSIBILITES DE DEVELOPPEMENT APRES IRRADIATION

Après irradiation du pôle postérieur de la blastula, jeune ou âgée, le développement embryonnaire se poursuit jusqu'à l'éclosion (90% des cas) sans que l'on puisse observer un retard.

L'élevage de 150 animaux issus d'œufs irradiés par 10 000 R au stade blastula jeune montre que la plupart d'entre eux meurent dès le premier stade larvaire, sans s'être nourris. Aucun adulte n'a pu être obtenu.

L'élevage de 200 animaux irradiés par 3 000 R a fourni 60 adultes ayant des gonades typiques, à l'exception de quatre d'entre eux. Ceux-ci, pourvus d'un tractus génital normal, présentaient des testicules de forme typique (haltère): leur diamètre était identique à la largeur du spermiducte, soit 0, 1 mm.

Les larves ayant à la naissance un petit nombre de gonies évoluent en adultes normaux, parfaitement féconds: il semble établi qu'il se produit une régulation au cours de la croissance larvaire.

Discussion

Deux hypothèses permettent d'expliquer les possibilités de survie extrêmement faibles des larves agamétiques:

a) Les cellules polaires de Leptinotarsa auraient des potentialités aussi étendues que chez les Diptères supérieurs: elles donneraient naissance, d'une part, aux gonies et, d'autre part, à certaines cellules de l'intestin moyen, à l'image de ce qui est connu chez Drosophila [13].

b) La destruction de la totalité des cellules polaires par les rayons X ne pourrait s'effectuer sans entraîner la destruction de cellules blastodermiques voisines. Cette seconde hypothèse est d'autant plus vraisemblable qu'il est nécessaire d'irradier toute la région polaire

postérieure: les cellules polaires ne se détachent pas en une masse, externe à l'œuf, comparable à celle observée chez Drosophila.

REFERENCES

[1] HEGNER, R. W., Biol. Bull. 16 (1908) 19.
[2] HEGNER, R. W., Biol. Bull. 20 (1911) 237.
[3] GEIGY, R., Revue Suisse Zool. 38 (1931) 187.
[4] ABOIM, A. N., Revue Suisse Zool. 52 (1944) 53.
[5] HAGET, A., C. r. Séanc. Soc. Biol. 147 (1953) 673.
[6] ALLEAUME, N., HAGET, A., P. V. Soc. Sci. Phys. Nat. de Bordeaux (1961) 92.
[7] HATAWAY, D. S., SELMAN, G. G., J. Embryol. exp. Morph. 9 (1961) 310.
[8] OELHAFEN, F., Arch. EntwMech. Org. 153 (1961) 120.
[9] JURA, C., Acta biol. cracov. 7 (1965) 89.
[10] IDRIS, B. E. M., Arch. EntwMech. Org. 152 (1960) 230.
[11] COUNCE, S. J., SELMAN, G. G., J. Embryol. exp. Morph. 3 (1955) 121.
[12] COUNCE, S. J., EDE, D. A., J. Embryol. exp. Morph. 5 (1957) 404.
[13] POULSON, D. F., WATERHOUSE, D. F., Aust. J. biol. Sci. 13 (1960) 541.
[14] ANDERSON, D. T., A. Rev. Ent. 11 (1966) 23.
[15] SCHNETTER, W., Arch. EntwMech. Org. 155 (1965) 637.

DISCUSSION

R. CAVALLORO: Do you have any data on the radiation sensitivity of colorado beetle eggs irradiated 'in toto'?

R. BLUZAT: Yes, I have results for the beginning of embryogenesis.

R. CAVALLORO: I should like to mention, if I may, that I have recently completed some work on the same species using X-ray equipment at 200 kV, 10 mA current, with a dose rate of 650 R/min, and found that of eggs given a dose of 1000 R at 25°C twenty-four hours after oviposition only 5.81% hatched, and that a dose of 2000 R caused total embryo mortality.

R. BLUZAT: Results obtained under my conditions of work are very close to yours, i.e. LD_{99} is between 600 and 800 R. On the other hand, the earlier developmental stages (from onset of intravitelline multiplication) are more resistant to radiation, i.e. $LD_{99} > 1000$ R. Moreover, in the latter case the larvae obtained are viable and normal.

G. LE MASNE: I should like to hear a little more on the phenomenon of larvae with a reduced number of gonia after irradiation treatment developing into perfectly fertile adults, as described in section 4.4 of your paper. Firstly, what do the gonads of the young larvae look like? Secondly, what sort of regulatory mechanism had you in mind? Is gonial multiplication resumed, and if so what factors come into play? It seems that this regulatory process would be worth studying.

R. BLUZAT: The gonads we saw in these irradiated larvae looked the same as those of the control insects. It would appear that regulation is not due to further multiplication of the gonia, but the processes involved have still not been elucidated.

V. LABEYRIE: Is it not possible that the mesodermic mass of the gonad is instrumental in inducing multiplication of gonia reduced appreciably in number by irradiation, since the number of gonia is normally a

function of a mass ratio of mesodermic cells to gonial cells in the early stages of development before differentiation into oögonia or spermatogonia?

R. BLUZAT: Indeed, it is highly likely that this ratio plays a part in gonia multiplication. We cannot rule out the possibility that the mesodermic tissue controls gonia multiplication more or less directly.

QUELQUES EFFETS DE L'IRRADIATION AUX RAYONS GAMMA SUR Spodoptera (Laphygma) exigua Hb. (LEPIDOPTERA: NOCTUIDAE)*

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Abstract — Résumé

SOME EFFECTS OF GAMMA IRRADIATION ON Spodoptera (Laphygma) Exigua Hb. (LEPIDOPTERA: NOCTUIDAE). A study has been made of the effect of gamma rays from a cobalt-60 source on the eggs, larvae, pupae and adults of Spodoptera exigua Hb., a lepidopteron which is harmful to crops in many climates. The insect was reared on an artificial diet based on powdered cabbage and wheat-germ. The irradiation of one- and four-day-old eggs with 3, 6 and 9 krad showed that none of the one-day-old eggs hatched, while the four-day-old ones gave 52, 18 and 0% of adults respectively. The larvae hatched from irradiated eggs showed retarded development, and the butterflies which developed were listless and of low fertility. Mature larvae irradiated at 3 and 5 krad give 100 and 22.5% of adults respectively; irradiation at 7 krad or more completely stops the production of adults. Irradiation of young pupae either kills them or leads to the hatching of malformed adults. Irradiation at 50 krad of pupae at the end of their development has no effect on hatching. The adults from nymphs irradiated at 50 krad show a fertility reduced to 0.5%, while the adults which have just hatched show total sterility after irradiation at the same dose. Higher doses reduce the frequency of mating, while females irradiated at 30 krad and mated with normal males lay infertile eggs.

QUELQUES EFFETS DE L'IRRADIATION AUX RAYONS GAMMA SUR Spodoptera (Laphygma) exigua Hb. (LEPIDOPTERA: NOCTUIDAE). L'effet des rayons gamma d'une source au cobalt-60 a été étudié sur les œufs, larves, pupes et adultes de Spodoptera exigua Hb., Lépidoptère nuisible aux cultures sous de nombreux climats. L'insecte était élevé sur milieu artificiel à base de poudre de chou et de germe de blé. L'irradiation d'œufs de 1 et 4 jours à 3, 6 et 9 krads montre qu'il n'y a aucune éclosion pour ceux de 1 jour et que ceux de 4 jours donnent 52, 18 et 0% d'adultes. Les larves issues d'œufs irradiés montrent un retard de développement et les papillons éclos sont sans vigueur et peu féconds. Les larves âgées irradiées à 3 et 5 krads donnent 100 et 22,5% d'adultes; l'irradiation à 7 krads et plus ne donne pas d'adultes. L'irradiation des pupes jeunes provoque une mortalité des pupes ou des éclosions d'adultes mal formés. L'irradiation à 50 krads de pupes en fin de développement n'affecte pas les éclosions. Les adultes issus de nymphes irradiées à 50 krads montrent une fertilité réduite à 0,5%. Les adultes venant d'éclore irradiés à 50 krads montrent une stérilité totale. Des doses plus élevées diminuent la fréquence des accouplements. Des femelles irradiées à 30 krads et accouplées à des mâles normaux pondent des œufs infertiles.

1. INTRODUCTION

Spodoptera exigua est une Noctuelle répandue dans le monde entier, surtout dans les régions tropicales, subtropicales et méditerranéennes. Sa chenille cause des dégâts parfois importants à des cultures très

* Travail effectué en 1966-1967 pendant un stage en France organisé par l'ASTEF (Association pour l'organisation des stages en France).

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variées, coton, tabac, vigne, luzerne, plantes maraîchères, etc. L'adulte est un migrateur pouvant effectuer des déplacements importants.

Une seule publication, à notre connaissance, donne les résultats d'études d'irradiations, qui ont été poursuivies en Union soviétique [1]. Etant donné l'importance économique de l'insecte, il nous a paru intéressant de reprendre cette étude de façon plus complète; nous verrons d'ailleurs que nos résultats ne concordent souvent pas avec ceux de Rasulov.

2. METHODE D'ELEVAGE

L'élevage de S. exigua sur milieu artificiel est assez facile. Différentes formules de milieu ont été mises au point [2, 3]; nous avons adopté une formule assez voisine, mise au point à la Station de zoologie de Montfavet par G. Guennelon et S. Poitout (Travaux non publiés), composée essentiellement de poudre de chou et de germe de blé, auxquels sont ajoutés les ingrédients qui composent habituellement un milieu semi-synthétique.

L'élevage des larves est réalisé sur ce milieu, qui est découpé en petits cubes et disposé dans des boîtes en matière plastique (de 17 × 10, 5 × 2, 5 cm). Chaque boîte contient environ une centaine de larves, qui sont élevées dans une pièce climatisée (25°C, 80% HR et éclairage de 18 h par tubes fluorescents). Dans ces conditions d'élevage, la préchrysalidation débute de 11^{e} jour. Dès le 10^{e} jour les boîtes contenant les larves sont placées dans un récipient plus vaste rempli au tiers de sable légèrement humidifié. Tous les jours, des larves qui ne sont pas entrées dans le sable sont placées dans un autre récipient avec du sable. La chrysalidation commence le 12^{e} jour et la séparation des sexes avant l'irradiation a été effectuée à ce stade. La durée de développement des nymphes est environ de 7 jours pour les femelles et de 8 jours pour les mâles.

3. CONDITIONS EXPERIMENTALES ET METHODES

Les irradiations ont été effectuées avec un irradiateur de ^{60}Co de 1400 Ci au Service de radio-agronomie du Centre d'études nucléaires de Cadarache. Le débit de dose était d'environ 1040 rad/min. La dosimétrie a été effectuée pour chaque exposition.

Pour l'accouplement des adultes, nous avons utilisé des boîtes en plastique de 8 cm de diamètre et 5 cm de hauteur, avec des couvercles aérés par un trou circulaire de 4 cm de diamètre et grillagé. Pour les contrôles de fécondité des adultes et de viabilité des œufs, on tapisse les parois de la cage avec du papier sulfurisé, sur lequel les papillons déposent leurs œufs. Tous les trois jours les adultes sont changés de cages, les pontes sont comptées. Un certain nombre de pontes sont gardées pour contrôler la viabilité des œufs, et les larves écloses sont comptées chaque jour.

On place un seul couple par cage, avec différentes combinaisons (mâle irradié × femelle non irradiée; femelle irradiée × mâle non irradié; témoin, mâle et femelle non irradiés). On installe 20 cages par com-

binaison. Les femelles sont disséquées après leur mort pour compter les spermatophores.

Ces cages sont placées dans une pièce climatisée (22°C, 80% HR) à la lumière naturelle. On utilise seulement les adultes qui ont éclos dans les 24 heures suivant l'irradiation. Les adultes sont nourris avec une solution d'eau et de miel à 10%.

4. RESULTATS

4.1. Irradiation des œufs

Les doses d'irradiation choisies ont été de 3, 6 et 9 krad, appliquées à des œufs de 1 et 4 jours (durée d'incubation 4,5 jours à 22°C).

Les œufs de 1 jour irradiés ne donnent aucune éclosion.

L'effet des rayons gamma sur les œufs de 4 jours est porté dans le tableau I. Nous voyons que, si les doses de 3 et 6 krad ne diminuent que peu le pourcentage d'éclosions, par contre l'effet de l'irradiation se fait sentir fortement sur le développement ultérieur, larvaire et nymphal, et l'éclosion d'adultes. Les larves issues d'œufs irradiés montrent un retard de développement; l'irradiation des œufs à 9 krad ne permet aucune formation d'adultes.

Proverbs et Newton [4] et Elbadry [5] attiraient déjà l'attention sur cette prolongation d'action de l'irradiation des œufs, respectivement sur Carpocapsa pomonella et Gnorimoschema operculella.

Nous ayons également examiné la fécondité et la fertilité des adultes issus d'œufs irradiés. Il est remarquable de constater (tableau II) que cette action est très marquée; en particulier, les adultes issus d'œufs irradiés à 6 krad présentent une viabilité d'œufs nulle, qu'il s'agisse des mâles ou des femelles. A cette dose, la longévité est assez courte et les adultes sont sans vigueur et peu féconds.

4.2. Irradiation des larves

L'irradiation des larves du dernier âge à différentes doses de 3 et 5 krad permet une évolution jusqu'aux adultes (tableau III).

Les adultes issus de larves irradiées à 3 et 5 krad montrent (tableau IV) une fertilité des œufs diminuée par rapport aux témoins, et une fécondité réduite.

4.3. Irradiation des nymphes

4.3.1. Eclosion

Les nymphes de 1 jour sont tuées par des doses de 10 krad. Les nymphes de 3 jours sont moins sensibles; une dose de 20 krad ne cause que 25% de mortalité des nymphes.

Des nymphes en fin de développement (7 jours pour les nymphes femelles et 8 jours pour les nymphes mâles) supportent des doses beaucoup plus fortes. Il faut atteindre 100 krad pour commencer à provoquer une mortalité sensible, et 120 krad pour empêcher toute formation d'adultes normaux.

TABLEAU I. EFFETS DU RAYONNEMENT GAMMA SUR LES ŒUFS AGES DE 4 JOURS DE Spodoptera exigua Hb.

(200 œufs par dose)

Dose (krad)	Œufs éclos (%)	Larves parvenues au dernier stade (%)	Larves mortes au dernier stade (%)	Nymphes mortes (%)	Œufs donnant des adultes (%)			Durée moyenne de développement de l'éclosion des œufs jusqu'à l'éclosion des adultes (j)
					Total	Mâle	Femelle	
0 (témoin)	95	96	5	2	84	47,6	52,4	20,9
3	87	76	8	15	52	47,7	52,3	24,1
6	86	63	18	59	18	63,6	36,4	27,5
9	65	13	92	100	0	0,0	0,0	0,0

TABLEAU II. NOMBRE D'ACCOUPLEMENTS, FECONDITE, FERTILITE ET LONGEVITE DES ADULTES DE Spodoptera exigua ISSUS DU CROISEMENT D'INSECTES NORMAUX AVEC DES INSECTES ISSUS D'ŒUFS IRRADIES

Dose (krad)	Nature des croisements		Pourcentage des femelles fécondées	Nombre d'accouplements par mâle	Nombre d'œufs par femelle	Œufs éclos (%)	Longévité des adultes (j)	
	Mâle	Femelle					Mâle	Femelle
0	N	N	100	2,60	611,7	87,5	10,3	9,6
3	I	N	95	1,45	395,9	51,8	9,7	9,4
3	N	I	80	2,60	313,7	28,4	10,3	9,9
6	I	N	5	0,05	232,0	0,0	5,8	10,7
6	N	I	45	0,45	116,4	0,0	10,5	5,0

N = non irradié , I = irradié.

TABLEAU III. EFFETS DU RAYONNEMENT GAMMA SUR LES LARVES DU DERNIER AGE DE Spodoptera exigua Hb.

(40 larves par dose)

Dose (krad)	Nombre de larves mortes	Nombre de nymphes mortes	Nombre d'adultes malformés	Nombre d'adultes normaux
0	0	0	0	40
3	0	0	0	40
5	0	4	27	9
7	9	9	22	0
10	28	12	0	0
15	33	7	0	0

TABLEAU IV. NOMBRE D'ACCOUPLEMENTS, FECONDITE, FERTILITE ET LONGEVITE DES ADULTES DE Spodoptera exigua Hb. ISSUS DU CROISEMENT D'INSECTES NORMAUX AVEC DES INSECTES ISSUS DE LARVES IRRADIEES

Dose (krad)	Nature des croisements		Pourcentage des femelles fécondées	Nombre d'accouplements par mâle	Nombre d'œufs par femelle	Œufs éclos (%)	Longévité des adultes (j)	
	Mâle	Femelle					Mâle	Femelle
0	N	N	90	2,15	647,9	90,1	13,7	12,4
3	I	N	80	1,30	645,6	60,5	14,5	16,3
3	N	I	80	1,35	187,8	33,9	17,0	13,3
5	I	N	30	0,45	584,3	24,1	11,7	15,9
5	N	I	40	0,50	110,5	13,6	17,8	8,9

N = non irradié, I = irradié.

4.3.2. Stérilité obtenue

Une dose de 5 krad appliquée à des nymphes de 1 jour donne des femelles à fécondité réduite et fertilité nulle; les mâles montrent une fertilité d'environ 42%.

Sur des nymphes de 3 jours la dose de 10 krad donne une fécondité et une fertilité nulles pour les deux sexes. Dans le cas des mâles irradiés à 10 krad, il n'y a d'ailleurs pas d'accouplements.

Des doses de 15 à 70 krad ont été appliquées à des nymphes de 7 jours (femelles) et de 8 jours (mâles). La fécondité des femelles diminue avec la dose mais reste encore de 25% par rapport au témoin à la dose de 70 krad (fig. 1). La fécondité des femelles normales accouplées à des mâles irradiés ne présente une légère diminution que pour 70 krad.

La fertilité des femelles irradiées décroît très vite en fonction de la dose d'irradiation pour devenir nulle à 30 krad (fig. 2). La fertilité des mâles décroît plus progressivement, et devient pratiquement nulle à partir de 50 krad.

Rasulov [1] indique avoir obtenu la stérilité de mâles issus de nymphes irradiées à 9 et 11 krad, et des femelles à 5 krad. Ces résultats sont évidemment très différents des nôtres. Nos résultats paraissent pourtant plus conformes à ce qui a été obtenu sur d'autres Lépidoptères de taille comparable, par exemple Ostrinia nubilalis Hb. [6], Pectinophora gossypiella [7] et sur des Lépidoptères de plus petite taille, par exemple Carpocapsa pomonella L. [8].

Des observations précises sur la vigueur sexuelle ont été effectuées en utilisant comme test le nombre d'accouplements successifs. Les irradiations jusqu'à 50 krad ne diminuent pas la vigueur sexuelle des mâles; la réceptivité des femelles irradiées paraît supérieure à celle des témoins.

4.3.3. Action résiduelle de doses substérilisantes

La dose de 45 krad appliquée à des nymphes mâles de 8 jours laisse une fertilité de 10% chez les mâles éclos. Il est intéressant d'examiner l'évolution et la descendance de ces œufs fertiles.

Le tableau V montre que les larves descendant de mâles irradiés (à l'état de nymphe) à 45 krad présentent une forte mortalité du premier et deuxième stade, à laquelle s'ajoute une mortalité au moment de la nymphose. Seulement 10% de ces larves donneront des adultes. Ces adultes eux-mêmes, qui constituent une génération F1, montrent une fertilité nulle (tableau VI).

Il y a donc là une action résiduelle très remarquable de l'irradiation. Si l'on regarde le résultat d'efficacité totale, on voit que la dose de 45 krad appliquée à des mâles provoque une extinction complète de la descendance malgré une fertilité de 10% des premiers œufs obtenus. Il serait intéressant de reprendre les mêmes observations aux doses inférieures.

Une action identique a été signalée sur d'autres Lépidoptères. Par exemple C. pomonella [8] et Paramyelois transitella [9].

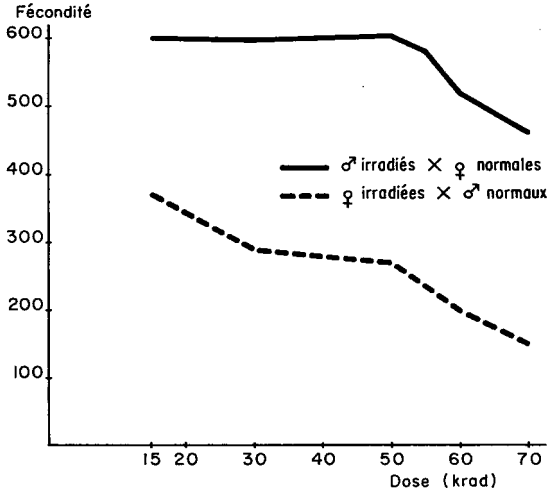

FIG. 1. Fécondité résultant de croisements insectes normaux × insectes issus de nymphes de 7 j (femelles) et 8 j (mâles) irradiées aux doses de 15 à 70 krad (la fécondité des témoins non irradiés était de 598 œufs).

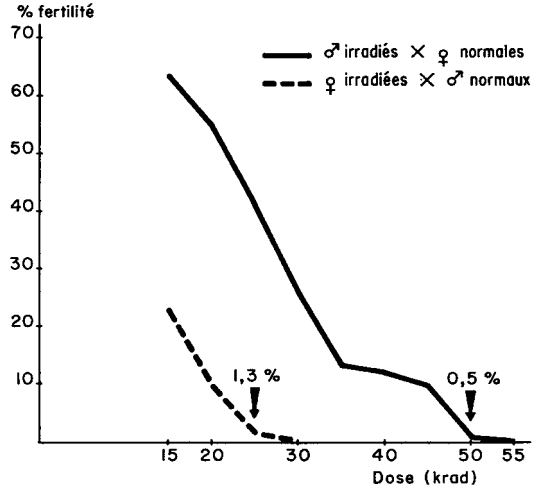

FIG. 2. Fertilité résultant de croisements insectes normaux × insectes issus de nymphes de 7 j (femelles) et 8 j (mâles) irradiées aux doses de 15 à 70 krad (la fertilité des témoins non irradiés était de 91%).

4.4. Irradiation des adultes

L'irradiation d'adultes dans la journée suivant leur éclosion a donné des résultats tout à fait comparables à ceux des irradiations de nymphes juste avant l'éclosion. La dose de 40 krad provoque la stérilité des femelles et laisse une fertilité de 11% chez les mâles. La dose de 50 krad est totalement stérilisante pour les deux sexes.

TABLEAU V. DEVELOPPEMENT DE LA DESCENDANCE DE NYMPHES IRRADIEES DE *Spodoptera exigua* Hb.
(MALE IRRADIE × FEMELLE NON IRRADIEE) A LA DOSE SUBSTERILISANTE

Nature des croisements	Œufs éclos[a] (%)	Larves mortes aux premier et deuxième stades (%)	Larves mortes au dernier stade (%)	Nymphes mortes (%)	Larves donnant des adultes F1 (%)		
					Total	Mâle	Femelle
Mâle non irradié × femelle non irradiée	91,0	4,0	5,0	2,0	89,0	47,6	52,4
Mâle irradié × femelle non irradiée	9,7	70,5	11,0	8,5	10,0	59,1	40,9

[a] 400 larves F1 par combinaison gardées pour l'élevage.

TABLEAU VI. NOMBRE D'ACCOUPLEMENTS, FECONDITE, FERTILITE ET LONGEVITE D'ADULTES F1 ISSUS DU CROISEMENT MALE IRRADIE A 45 krad × FEMELLE NON IRRADIEE

Nature des croisements	Pourcentage des femelles fécondées	Nombre d'accouplements par mâle	Nombre d'œufs par femelle	Œufs éclos (%)	Longévité des adultes (j)	
					Mâle	Femelle
Mâle non irradié × femelle non irradiée	100	2,60	611,7	87,5	10,3	9,6
Mâle F1 × femelle non irradiée	38,9[a]	0,54	375,6	0,0	7,8	13,8
Mâle non irradié × femelle F1	66,7[a]	0,89	256,7	0,0	12,7	7,6

[a] 18 cages par combinaison.

REMERCIEMENTS

L'auteur remercie M. Féron d'avoir facilité la réalisation et la rédaction de ce travail dans son laboratoire. Il remercie le Gouvernement pakistanais et le Gouvernement français d'avoir permis l'organisation et le bon déroulement de ce stage.

REFERENCES

[1] RASULOV, F.K., Khlopkovodstvo 7 (1963) 41-42.
[2] BOT, J., J. ent. Soc. sth. Afr. 29 (1967) 206-09.
[3] SHOREY, H.H., HALE, R.L., J. econ. Ent. 58 (1965) 522-24.
[4] PROVERBS, M.D., NEWTON, J.R., Can. J. Zool. 40 (1962) 401-20.
[5] ELBADRY, E., Ann. ent. Soc. Am. 58 (1965) 206-09.
[6] ANWAR, M., « Stérilisation de la pyrale du maïs (Ostrinia nubilalis Hb.) par l'irradiation aux rayons gamma », ces comptes rendus, SM-102/15.
[7] OUYE, M.T. et al., J. econ. Ent. 57 (1964) 387-90.
[8] PROVERBS, M.D., NEWTON, J.R., Can. Ent. 94 (1962) 1162-70.
[9] HUSSEINY, M.M., MADSEN, H.F., Hilgardia 36 (1964) 113-37.

DISCUSSION

C.F. CURTIS: I was interested to note the high doses of ionizing radiation required to sterilize these Lepidoptera, in comparison with the doses required for Diptera. Could you suggest any explanation for this great difference?

M. ANWAR: Nothing is really known about why some insect species are more radiosensitive than others. Probably the size of the organism, its metabolism, and the number and size of chromosomes are all contributing factors.

H. ERDMAN: Can Spodoptera exigua be mass-reared? With what numbers were you working?

M. ANWAR: I did not need many insects for my preliminary studies. I always reared them in groups of about 100 larvae per rearing cage, on an artificial medium, and used to obtain between 80 and 90% adults, which shows that it is quite feasible to mass-rear them.

M. COHEN: Since S. exigua seems to be easily reared, do you think it would be feasible to apply the irradiation technique to control of this insect in the field and in view of your laboratory results would sterilization of females be preferable to sterilization of males?

M. ANWAR: We did experiments under laboratory conditions with populations of (a) pupae irradiated at a late stage in their development with a dose of 50 krad and (b) normal pupae, mixed in the following proportions: 1 normal pair for every 5 sterile pairs; 1 normal pair for every 5 sterile males; 1 normal pair for every 5 sterile females. From the results of these experiments we found very great variations in the viability of eggs laid by the females. This showed that introduction of sterile males appreciably reduced egg viability, but an even greater decrease was noted on simultaneous introduction of sterile males and females; it would thus appear that sterile females could be used in the same way as the males to increase the chances of success in an eradication programme.

R. BLUZAT: Do you know the number of chromosomes S. exigua has and have you compared this with the number of chromosomes possessed by creatures exhibiting a radiation resistance of the same order?

M. ANWAR: I'm afraid the answer on both scores is 'no'.

J.E. SIMON: Was there any cannibalism amongst Spodoptera larvae during mass rearing?

M.ANWAR: I have never observed any.

J.E. SIMON: Were the larvae reared together or separately?

M.ANWAR: They were always reared in groups.

SENSIBILITE DES DIVERS STADES DE DEVELOPPEMENT DE *Sitophilus zeamais* MOTS (*S. oryzæ* L.) AUX RADIATIONS IONISANTES

Etude des stades endogés par radiographie et enregistrement actographique

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(Présenté par P. Pélegrin)

Abstract — Résumé

RADIOSENSITIVITY OF *Sitophilus zeamais* MOTS (*S. oryzae* L.) AT VARIOUS STAGES OF DEVELOPMENT: A STUDY OF THE LARVAL STAGES BY MEANS OF RADIOGRAPHY AND 'ACTOGRAPHIC' RECORDING. The paper describes an original method of study, based on the employment of an 'actograph' which records the vitality of the larval stages and makes it possible to study the larva population inside grains (actograph for electro-acoustic detection developed by the INRA Acoustic Physiology Laboratory, Jouy-en-Josas). The contamination rate and the death rate of the larvae are observed by radiography. The effects of irradiation at doses of 2000, 4000, 8000 and 16 000 rad are studied in the egg stage, on larvae aged 5-7 days, 13-15 days and 19-21 days and on adults, whose fertility is measured 0-5 days, 5-10 days and 10-15 days after irradiation. The effects of irradiation on the second generation bred from the various stages irradiated are noted.

SENSIBILITE DES DIVERS STADES DE DEVELOPPEMENT DE *Sitophilus zeamais* MOTS (*S. oryzæ* L.) AUX RADIATIONS IONISANTES: ETUDE DES STADES ENDOGES PAR RADIOGRAPHIE ET ENREGISTREMENT ACTOGRAPHIQUE. Les auteurs présentent une méthode d'étude originale, fondée sur l'emploi d'un appareillage actographique, qui enregistre et traduit la vitalité des stades endogés et permet d'apprécier la population larvaire à l'intérieur des grains (actographe à détection électro-acoustique mis au point par le Laboratoire de physiologie acoustique de l'INRA, Jouy-en-Josas). Le contrôle du taux de contamination et de la mortalité larvaire se fait par radiographie. Les auteurs ont étudié les effets de l'irradiation (doses de 2000, 4000, 8000 et 16 000 rad) sur le stade œuf, sur les larves âgées respectivement de 5 à 7 jours, 13 à 15 jours, 19 à 21 jours et sur les adultes (fécondité 0 à 5 jours, 5 à 10 jours, 10 à 15 jours après l'irradiation); ils ont également fait des observations sur les conséquences de l'irradiation sur la seconde génération issue des divers stades irradiés.

Divers auteurs [1-4] ont déjà utilisé des techniques de détection acoustique ou les observations radiographiques pour étudier le développement à l'intérieur des grains de céréales de divers insectes des denrées. Nous utilisons conjointement ces deux techniques en vue de préciser la sensibilité aux radiations ionisantes des stades endogés du charançon des grains.

L'enregistrement de l'activité de ces divers stades, avant et après irradiation, est obtenu grâce à un détecteur électro-acoustique associé à un amplificateur très puissant, appareil mis au point par R. G. Busnel[1].

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Nous avons limité cette étude à Sitophilus zeamais Mots. L'essentiel de nos résultats est en accord avec les travaux antérieurement publiés par divers chercheurs et par nous-mêmes [5-8].

1. MATERIEL ET METHODE

L'élevage de S. zeamais a été réalisé sur des grains de blé, dans une étuve, avec une humidité relative de 75% ± 5% et une température constante de 27° C ± 1° C.

Cinquante grains de blé, préalablement bien conditionnés (HR et température indiquées ci-dessus) sont alors mis dans des petits tubes de matière plastique, en présence de 10 couples de charançons, âgés de 1 à 3 jours. Pour chaque expérience on prépare cinq répétitions d'un tel échantillon. Les grains ainsi soumis à l'oviposition furent conservés dans les conditions optimales précitées, en assurant en particulier une humidité relative favorable en stockant les tubes de ponte dans un exsiccateur dont l'humidité était maintenue constante par une solution de potasse. Le temps de ponte des adultes était limité à 24 h ou 48 h, afin d'avoir ensuite une population larvaire d'âge uniforme. Connaissant bien, dans ces conditions, la durée relative des divers stades (œufs, larves et nymphes), il nous était facile de disposer au moment voulu de lots homogènes pour les irradier.

La source d'irradiation utilisée était un irradiateur γ de 3000 Ci de ^{60}Co, dans lequel la source était disposée selon les génératrices d'un cylindre. La température dans l'enceinte d'irradiation était de 27° C ± 0,5° C. Pour les divers stades expérimentés, quatre doses différentes ont été utilisées: 2000, 4000, 8000 et 16 000 rad, administrées respectivement en des temps de 1 min 43 sec, 3 min 26 sec, 6 min 52 sec et 13 min 44 sec, en rapport avec l'intensité de rayonnement de la source, qui est de 70 krad/h.

L'activité des larves dans les grains était enregistrée pour l'ensemble des 50 grains de chaque lot, d'une part avant l'irradiation, d'autre part à des intervalles de temps réguliers après l'irradiation. La date d'apparition des adultes et leur nombre furent notés. Les adultes issus des lots irradiés furent alors mis à nouveau dans des conditions favorables à la ponte sur de nouveaux grains pendant 22 jours. L'activité de leur progéniture fut évaluée par actographie et le nombre d'adultes de deuxième génération issus de ces grains fut compté. Les lots de grains irradiés ou ceux servant au développement de la deuxième génération furent, en fin d'expérience, examinés par radiographie, pour apprécier le taux de mortalité des stades endogés ou le pourcentage de grains attaqués.

2. RESULTATS ET DISCUSSION

2.1. Sensibilité des œufs

La procédure expérimentale fut la suivante: 10 couples de Sitophilus zeamais (âgés de 3 jours) furent mis, pour la ponte, en présence de 50 grains de blé le 21 mai 1966 (5 lots); les adultes furent retirés du blé le 23 mai et quatre lots furent irradiés à 2000, 4000, 8000 et 16 000 rad (le 23 mai également), l'âge des œufs au moment de l'irradiation étant de 2 jours

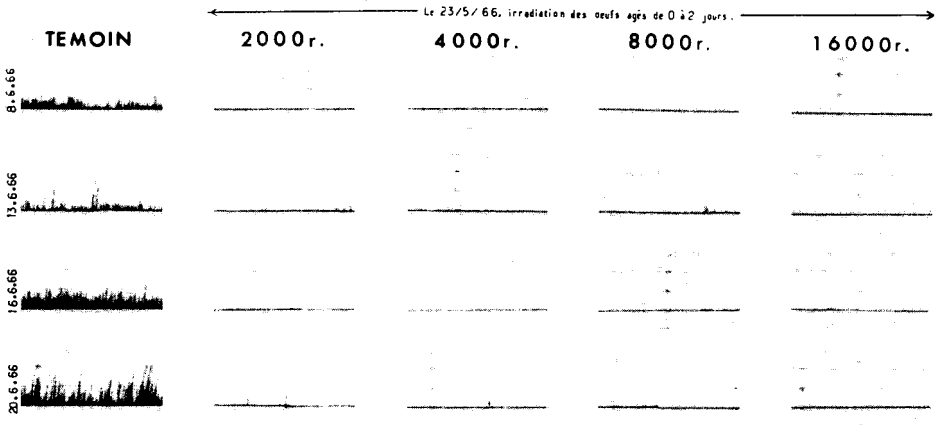

FIG. 1. Actographies de lots de 50 grains de blé ayant reçu la ponte de 10 couples de S. zemais pendant 48 h. Irradiation des grains avec les œufs inclus, ces derniers étant au plus âgés de 2 jours. Actographies enregistrées 16, 21, 24 et 28 jours après l'irradiation.

au maximum; les activités larvaires dans chacun des lots irradiés et dans le lot témoin furent enregistrées les 8, 13, 16 et 20 juin (les larves étant donc âgées de 16 à 30 jours).

La figure 1, qui reproduit les portions des actogrammes obtenus dans ces conditions, montre qu'aucun des lots irradiés n'hébergeait de larves actives. Le lot témoin, par contre, montrait une activité larvaire qui allait en s'amplifiant avec l'âge. Une dose d'irradiation de 2000 rad suffit donc pour empêcher le développement de la ponte de Sitophilus zeamais.

L'examen des radiographies révèle les piqûres de ponte, avec un petit nombre de débuts de galeries alimentaires, témoignages d'éclosions, dans les lots irradiés à 2000, 4000 et 8000 rad. A 16 000 rad aucune galerie n'est décelable, il n'y a donc eu aucune éclosion des œufs.

2.2. Sensibilité des larves

a) Larves âgées de 5 à 7 jours

La procédure expérimentale fut la suivante: ponte (comme ci-dessus) du 9 au 11 mai, incubation, puis irradiation le 23 mai; actographie avant irradiation le 23 mai, après irradiation du 24 mai au 10 juin; contamination pendant 22 jours de nouveau blé par les adultes issus du lot témoin et des lots irradiés à 2000 et 4000 rad; actographie des larves de deuxième génération du 8 au 13 août.

La figure 2 donne les résultats de cette expérience. On notera que l'actographie de contrôle, sur les divers lots avant l'irradiation, révèle une activité faible en raison du très jeune âge des larves. Cette activité va s'accroître régulièrement avec l'âge dans le lot témoin et les lots irradiés à 2000 et 4000 rad. Mais pour les lots irradiés à 8000 et 16 000 rad, il est évident que le développement larvaire est définitivement stoppé vers l'âge de 20 à 22 jours. Il suffit donc d'une dose de 8000 rad pour tuer dans un délai maximal de 15 jours des larves âgées de 5 à 7 jours.

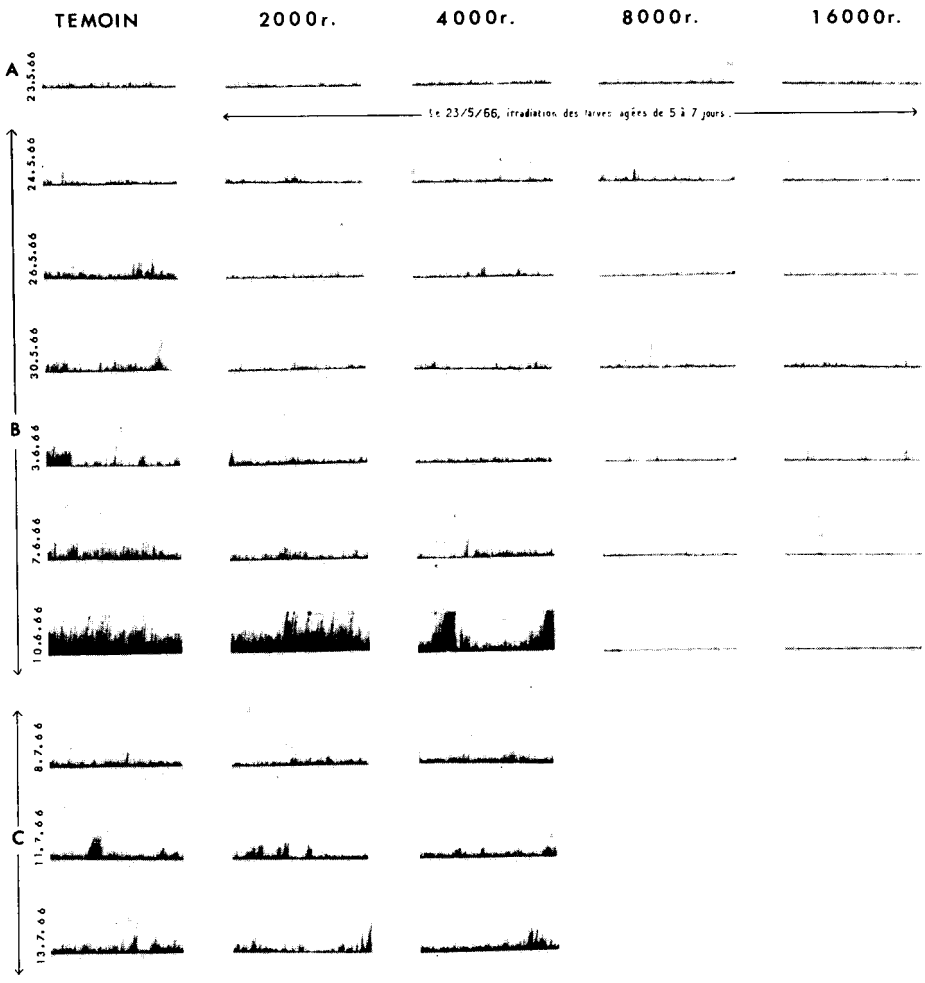

FIG. 2. Actographies de populations larvaires dans 50 grains de blé, contaminés par 10 couples de Calandres, pendant 48 h. Irradiation des larves à l'âge de 5 à 7 jours. A, actographies enregistrées avant l'irradiation; B, enregistrements effectués 1, 3, 7, 11, 18 et 21 jours après l'irradiation; C, actogrammes de la deuxième génération larvaire provenant des adultes éclos du lot témoin et des lots irradiés à 2000 et 4000 rads.

La suite de l'expérience (fig. 2C) portant sur les adultes issus des lots irradiés à 2000 et 4000 rad montre que ces derniers sont féconds et donnent une progéniture larvaire active.

Après radiographie des divers lots en fin d'expérience, on peut dénombrer les grains attaqués, ce qui devrait nous permettre d'évaluer la mortalité larvaire, en tenant compte du nombre d'adultes éclos. Toutefois, nous constatons que, dans le lot témoin, pour 44 grains attaqués (sur 50), on n'a que 21 adultes, ce qui représente déjà une mortalité importante. Elle est due en partie au fait que le même grain héberge souvent deux larves, parfois trois. En outre, nous avons décelé quelques acariens prédateurs (Pediculoides ventricosus). Il serait sans doute possible de

décompter les mortalités dues à ce prédateur, mais il nous paraît suffisant de comparer les radiographies, qui montrent manifestement que dans les lots irradiés à 4000, 8000 et 16 000 rad les galeries larvaires sont moins étendues que dans le lot témoin, signe d'une mortalité d'autant plus précoce que la dose reçue est plus forte. Cette mortalité est simplement estimée de façon valable en indiquant le nombre d'adultes éclos en regard du nombre de grains attaqués. Elle est manifestement en rapport avec l'irradiation dès une dose de 4000 rad.

	Témoin	2000	4000	8000	16 000 rad
Grains attaqués	44	35	35	41	40
Adultes éclos	21	20	2	0	0

De même, les radiographies de la deuxième génération larvaire montrent que les adultes issus de larves irradiées demeurent féconds même pour le lot irradié à 4000 rad. Mais les données de l'expérience, où l'on compare la descendance d'un nombre variable de couples, ne permettent pas de préciser si cette fécondité a subi une diminution chez les adultes issus des lots irradiés. Toutefois les chiffres obtenus soulignent suffisamment la fertilité de ces insectes:

	Témoin	2000	4000 rad
Grains attaqués	49	49	40
Adultes éclos	52	46	30

Les dates d'émergence des premiers adultes dans les lots irradiés concordent avec celles des lots témoins, mais les dernières émergences sont plus tardives. Dans la première génération, on relève les dates du 15 au 22 juin pour le lot témoin, du 15 au 29 juin pour le lot irradié à 2000 rad. Dans la deuxième génération, on note respectivement: du 18 juillet au 9 août pour le lot témoin, du 18 juillet au 22 août pour le lot à 2000 rad, du 18 juillet au 30 août pour le lot à 4000 rad. Un retard du développement est donc plus manifeste dans la deuxième génération.

b) Larves âgées de 13 à 15 jours

La procédure fut la même que précédemment: ponte du 9 au 11 mai; actographie avant irradiation les 25 et 31 mai; irradiation le 31 mai (larves âgées de 13 à 15 jours); actographie après irradiation du 1er au 8 juin; contamination de nouveau blé par les adultes issus des divers lots pendant 22 jours; actographie des larves de deuxième génération du 8 au 13 juillet.

L'activité des larves avant irradiation (fig. 3) est beaucoup plus grande que dans l'expérience précédente, en raison de leur développement plus avancé. L'actographie après irradiation révèle que la dose de 16 000 rad stoppe presque immédiatement l'activité larvaire. Par contre, le développement s'effectue jusqu'à l'éclosion d'imagos dans les lots irradiés à 2000, 4000 et 8000 rad. Toutefois, la comparaison des actogrammes révèle une activité un peu moins forte que chez les témoins, conséquence d'une certaine mortalité larvaire et d'une perte de vitalité. Mais on ne décèle un retard dans l'éclosion des imagos que dans le lot irradié à 2000 rad pour lequel les apparitions d'imagos s'étalent du 15 juin au

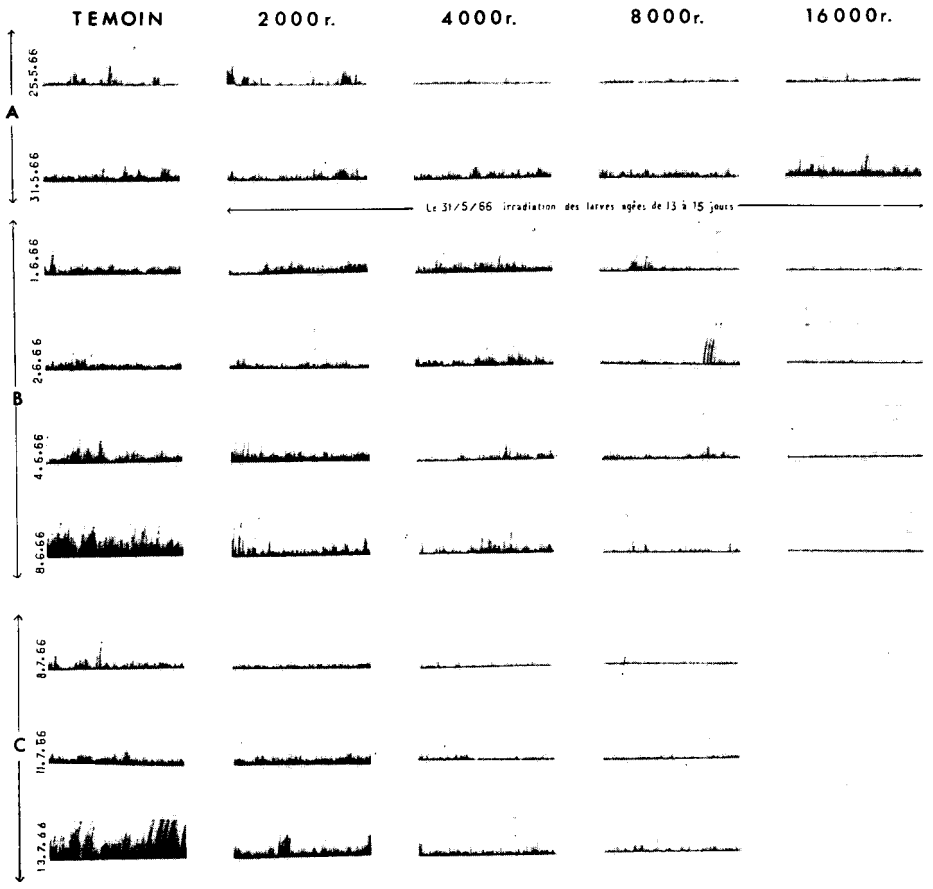

FIG. 3. Irradiation de larves âgées de 13 à 15 jours. A, actographie des populations larvaires avant irradiation; B, enregistrements effectués 1, 2, 4 et 8 jours après l'irradiation; C, actogrammes de la deuxième génération larvaire engendrée par les adultes issus du lot témoin et des lots irradiés à 2000, 4000 et 8000 rads. Noter que, dans ces derniers lots, l'activité des larves est moins forte: retard du développement et de l'émergence des adultes, associé à une mortalité plus forte.

7 juillet, alors que pour tous les autres lots (témoins et irradiés) cette période va du 15 au 22 juin.

L'examen des radiographies après la fin des éclosions imaginales révèle les proportions suivantes entre le nombre de grains attaqués et le nombre d'imagos sortis:

	Témoin	2000	4000	8000	16 000 rad
Grains attaqués	26	34	36	30	28
Adultes éclos	26	16	6	2	0

Ces radiographies montrent que, dans les lots irradiés, plusieurs imagos sont morts à l'intérieur des grains. En particulier, on peut en dénombrer huit dans le lot à 2000 rad. Ce fait pourrait traduire une

certaine déficience des insectes et expliquer par ailleurs le retard des éclosions. Par contre, dans les lots irradiés à 4000 et 8000 rad, on décèle surtout une mortalité larvaire, les galeries étant inachevées et moins développées, surtout dans le lot à 8000 rad.

Les adultes issus du lot témoin et des lots irradiés furent transférés sur du grain sain, pendant 22 jours. La seconde génération des divers lots donne respectivement 47 (lot témoin), 45 (lot à 2000 rad), 27 (lot à 4000 rad) et 20 imagos (lot à 8000 rad). Les actographies des populations larvaires correspondantes révèlent une activité plus ou moins forte, qui témoigne d'un retard dans l'évolution des larves issues des lots irradiés. En effet, alors que les éclosions d'imagos dans le lot témoin et dans le lot à 2000 rad s'étalaient du 18 juillet au 22 août, elles ont été notées du 18 juillet au 31 août pour le lot à 4000 rad, et du 9 au 30 août pour celui à 8000 rad.

En outre, la radiographie des populations larvaires de deuxième génération révèle une mortalité croissante avec les doses d'irradiation reçues par la génération larvaire parentale. On note en effet:

	Témoin	2000	4000	8000 rad
Grains attaqués	48	50	39	42
Adultes éclos	47	45	27	20

On peut donc conclure que les doses d'irradiation intermédiaires entre 8000 et 16 000 rad doivent entraîner 100% de mortalité dans une population de larves âgées de 13 à 15 jours. Les adultes issus des larves irradiées à 2000, 4000 et 8000 rad demeurent féconds, mais leur descendance subit un retard important dans son développement et présente une mortalité plus grande que les témoins.

c) Larves âgées de 19 à 21 jours

La procédure expérimentale fut la même que dans les expériences précédentes. La figure 4A montre que l'activité larvaire avant irradiation était tout à fait comparable dans les divers lots. Après irradiation (fig. 4B), cette activité reste apparemment normale dans les divers lots, mais aucun adulte n'éclot du lot irradié à 16 000 rad. Le nombre d'adultes issus des autres lots est respectivement de 28 (lot témoin), 24 (2000 rad), 21 (4000 rad) et 13 (8000 rad). Mais nous n'avons pas obtenu pour ces lots de documents radiographiques corrects, par suite d'un incident technique, et n'avons pu, en conséquence, rapporter le nombre d'éclosions au nombre de grains attaqués.

Les dates d'émergence des imagos s'étalent du 15 au 22 juin pour tous les lots, sauf pour le lot à 2000 rad, pour lequel on note, comme dans les expériences précédentes, certaines sorties tardives du 15 au 29 juin.

Ces adultes, mis à contaminer du blé sain pendant 22 jours, ont donné des populations larvaires de deuxième génération dont nous reproduisons les actogrammes respectifs (fig. 4C). L'activité demeure faible pour les lignées issues des lots irradiés à 4000 et 8000 rad, et présente un net retard par rapport au lot témoin. Le nombre d'adultes issus de ces divers lots a été respectivement de 50 (lot témoin), 30 (2000 rad) et 12 (4000 rad); le lot à 8000 rad n'a donné aucun adulte. Les dates

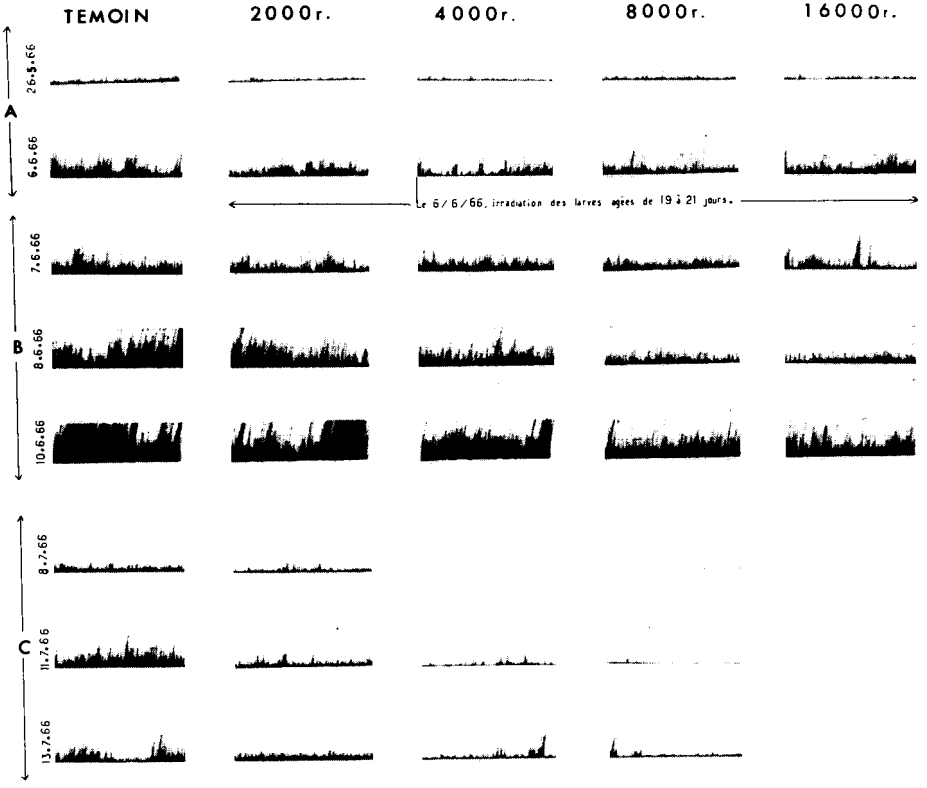

FIG. 4. Irradiation de larves âgées de 19 à 21 jours. A, actogrammes avant l'irradiation; B, après l'irradiation; C, actogrammes des populations larvaires de deuxième génération engendrées par les adultes issus des lots irradiés et du lot témoin. Comparer la diminution croissante d'activité dans les lots à 2000, 4000 et 8000 rads avec les données fournies par la radiographie.

d'émergence furent respectivement du 18 juillet au 22 août (lot témoin), du 1er août au 3 septembre (2000 rad) et du 9 août au 3 septembre (4000 rad).

L'examen des radiographies de cette deuxième génération larvaire montre que le nombre de grains présentant des galeries larvaires est très comparable chez les témoins et chez le lot à 2000 rad (respectivement 42 et 45 grains attaqués). Mais dans le lot à 4000 rad, il n'y a que 14 grains présentant des galeries avancées, et deux seulement dans le lot à 8000 rad. Par contre, on observe un grand nombre de piqûres de ponte, non associées à des galeries, dans le lot à 4000 rad, et peu de piqûres de ponte dans le lot à 8000 rad.

Il est évident que les adultes issus des larves irradiées à 4000 et 8000 rad sont peu féconds ou peu fertiles. En particulier, les adultes du lot à 4000 rad, s'ils laissent d'assez nombreuses piqûres de ponte, doivent déposer des œufs en majeure partie stériles. On peut penser qu'il s'agit là d'effets de mutations létales dominantes, produites par l'irradiation.

2.3. Sensibilité des adultes – Effets de l'irradiation sur la fécondité

Après avoir soumis des adultes à des doses d'irradiation de 2000, 4000, 8000 et 16 000 rad, nous les avons mis pondre dans des lots de grain sain. Chaque expérience portait sur 10 couples disposant de 50 grains de blé. Tous les cinq jours, ces insectes étaient transférés dans un nouveau milieu, afin d'apprécier leur fertilité 0 à 5 jours, 5 à 10 jours, 10 à 15 jours et 15 à 20 jours après l'irradiation.

L'activité des populations larvaires obtenues dans les divers lots fut enregistrée à plusieurs reprises au cours de leur développement (tous les 3 ou 4 jours); le nombre d'adultes éclos et les dates de leur émergence ont été notés; enfin, les lots de grains furent radiographiés après la sortie des derniers imagos.

Les adultes issus de cette première génération furent mis pour la ponte dans de nouveaux milieux, pour comparer leur fertilité.

a) Descendance issue des pontes déposées 0 à 5 jours après l'irradiation

Les actogrammes (fig. 5) révèlent l'absence d'activité larvaire dans les lots d'adultes irradiés à 8000 et 16 000 rad. Le nombre d'imagos issus des autres lots sont respectivement: 16 (lot témoin), 14 (2000 rad) et 9 (4000 rad). Les dates d'émergence s'étalent du 1er au 7 juillet pour les témoins, et du 1er au 12 juillet pour les lots irradiés. Les radiographies montrent une surpopulation des grains dans le lot témoin, ce qui explique une mortalité notable (50 grains attaqués ne donnent que 16 adultes), mortalité imputable aussi partiellement à quelques acariens prédateurs (Pediculoides ventricosus). Ce prédateur se retrouve aussi dans les lots irradiés, mais il demeure évident que le nombre de grains ne présentant pas de galeries larvaires s'acroît avec la dose d'irradiation. Le nombre de grains attaqués par les différents lots fut de 38 (2000 rad), 19 (4000 rad) et 0 (8000 et 16 000 rad). Cependant, le nombre de grains présentant des piqûres de ponte non associées à des galeries larvaires est très élevé dans ces lots irradiés; ils sont particulièrement apparents dans le lot à 4000 rad et dans le lot à 16 000 rad.

On peut en conclure que, dès 4000 rad, un certain nombre d'œufs ne sont pas féconds, et que tous sont stériles après irradiation à 8000 et 16 000 rad. Les adultes issus des lots à 2000 et 4000 rad purent donner une descendance larvaire (deuxième génération), comme en témoignent les actogrammes (fig. 5C). Le nombre d'adultes de deuxième génération fut respectivement de 44 (témoins), 40 (2000 rad) et 30 (4000 rad). A nouveau, les dates d'émergence, du 9 août au 2 septembre dans les lots irradiés, présentent un certain retard par rapport au témoin (du 9 au 28 août). Les radiographies des grains ayant hébergé cette deuxième génération montrent dans les trois lots de très nombreuses galeries larvaires. Mais on note des galeries larvaires qui s'arrêtent en cours de développement, témoignage d'une certaine mortalité.

b) Descendance issue des pontes déposées 5 à 10 jours après l'irradiation

A nouveau, dans cette expérience, seuls les lots à 2000 et 4000 rad donnent naissance à des adultes, en petit nombre d'ailleurs: 2 (4000 rad) et 6 (2000 rad), contre 44 (témoin). Le lot à 8000 rad présente un acto-

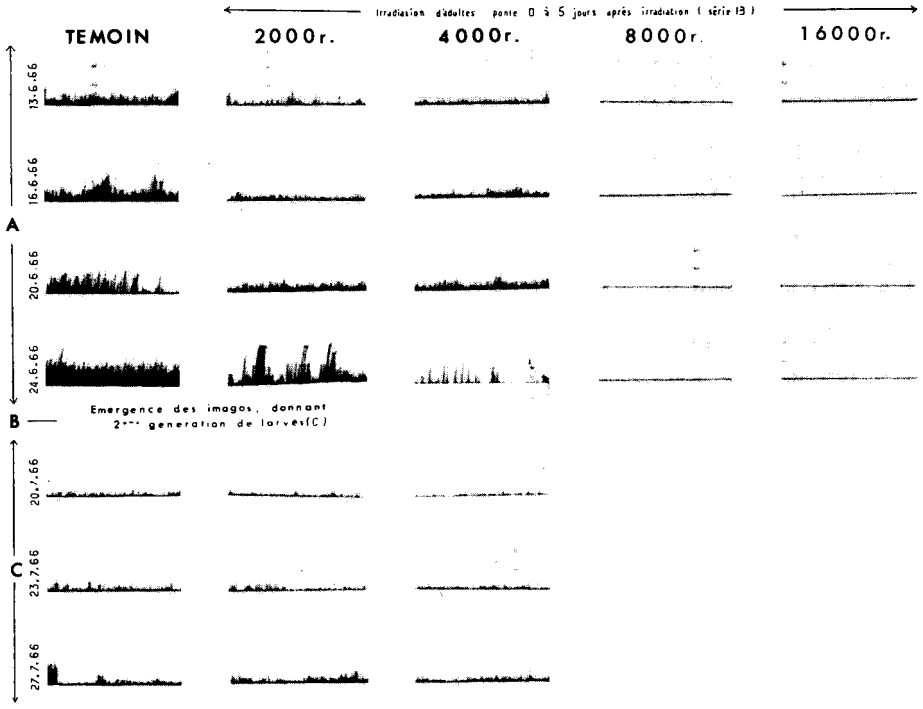

FIG. 5. Irradiation d'adultes: ponte 0 à 5 jours après l'irradiation, A, actogrammes des populations larvaires enregistrés à diverses périodes du développement; B, émergence des adultes de deuxième génération; C, actogrammes des populations larvaires de deuxième génération.

gramme légèrement positif (fig. 6A) et la radiographie ultérieure des grains révèle au plus deux galeries larvaires inachevées. L'émergence des adultes dans le lot témoin s'étale du 1er au 12 juillet, alors qu'elle se prolonge jusqu'au 3 août dans le lot à 2000 rad. Les radiographies montrent un grand nombre de piqûres de ponte dans le lot à 4000 rad, celles-ci étant moins nombreuses dans les lots à 8000 et 16 000 rad. Ces piqûres non accompagnées de galeries larvaires témoignent selon toute vraisemblance d'une mortalité importante au stade « œuf » (œufs inféconds).

La deuxième génération larvaire est peu abondante dans les lots à 2000 et 4000 rad, comme le révèlent les actogrammes (fig. 6C) et comme le confirment les radiographies. Les émergences d'adultes ont été respectivement de 59 (témoin), 15 (2000 rad) et 1 (4000 rad). Dans le lot à 2000 rad, les radiographies font apparaître une certaine mortalité tardive: 4 imagos morts dans le grain. Par contre, il ne semble pas y avoir de mortalité au stade « œuf » ou « jeune larve » (19 grains attaqués: 15 imagos vivants + 4 imagos morts).

c) Descendance issue des pontes déposées 10 à 15 jours après l'irradiation

Les actogrammes (fig. 7) révèlent une activité larvaire très faible et temporaire (début du développement) dans les lots irradiés à 2000,

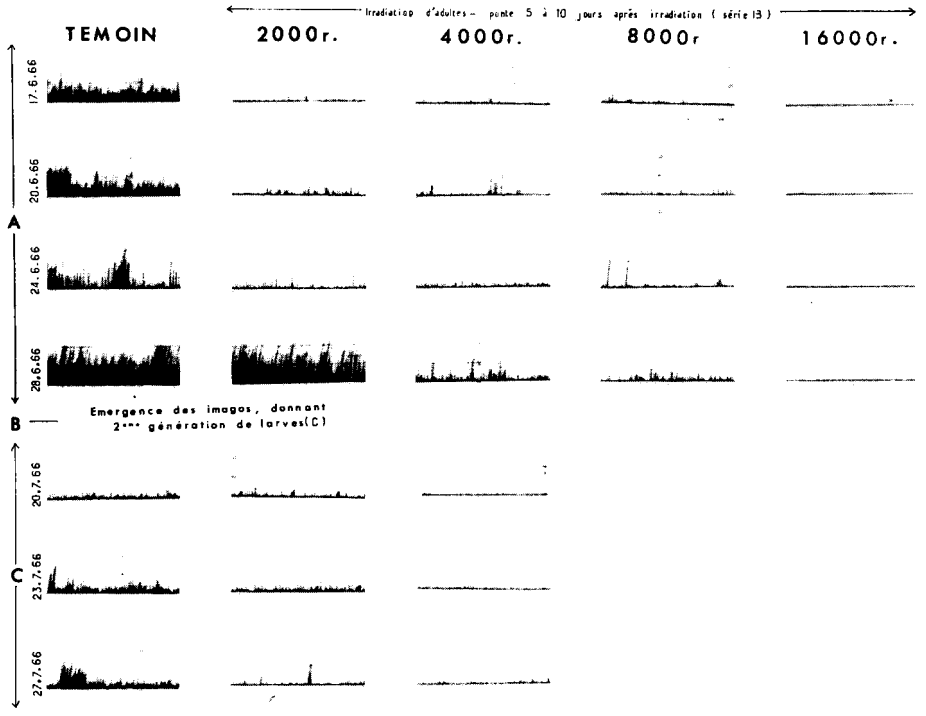

FIG. 6. Irradiation d'adultes: ponte 5 à 10 jours après l'irradiation. A, actogrammes des populations larvaires enregistrés à diverses périodes du développement; B, émergence des adultes de deuxième génération; C, actogrammes des populations larvaires de deuxième génération.

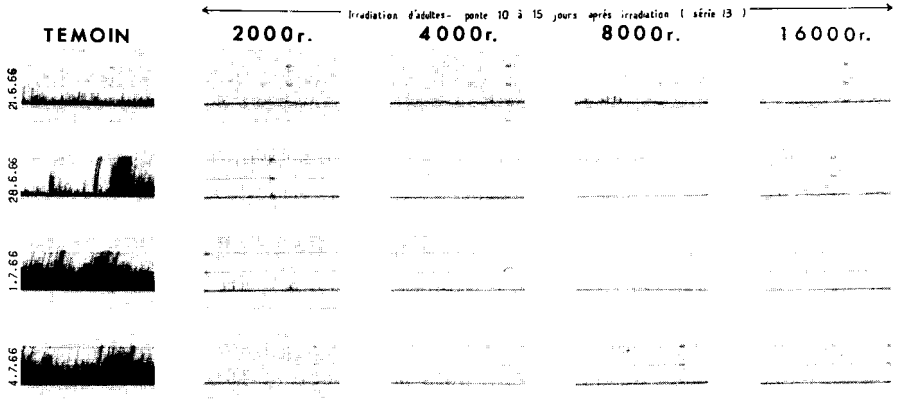

FIG. 7. Irradiation d'adultes: ponte 10 à 15 jours après l'irradiation. Actogramme des populations larvaires. Une très faible activité temporaire se manifeste dans les lots à 2000, 4000 et 8000 rads. Aucun des lots irradiés n'a donné de descendance viable.

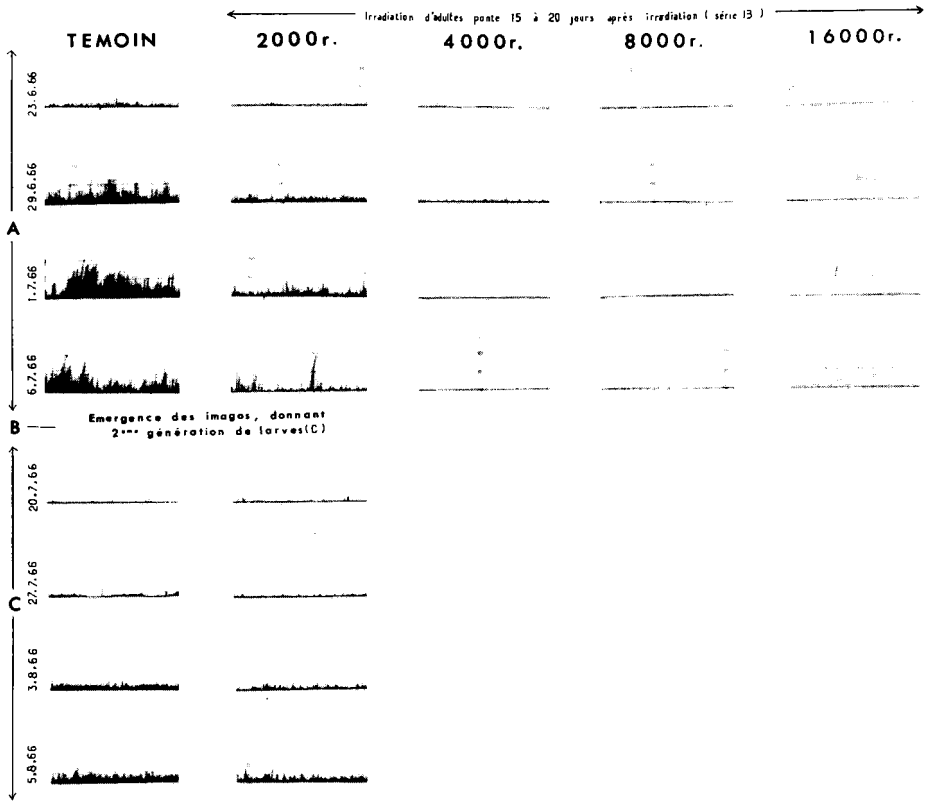

FIG. 8. Irradiation d'adultes: ponte 15 à 20 jours après l'irradiation. A, actogrammes des populations larvaires; B, émergence des adultes de deuxième génération pour le lot témoin et le lot à 2000 rads; C, actogrammes des populations larvaires de deuxième génération.

4000 et 8000 rad. En fait, aucun adulte n'est sorti de ces lots. Les radiographies montrent de rares galeries larvaires, jamais achevées, mais par contre de nombreux grains avec des piqûres de ponte, en particulier dans les lots à 2000 et 4000 rad, 10 à 15 jours après l'irradiation, le taux de fertilité des œufs est donc encore plus faible que dans l'expérience précédente.

d) Descendance larvaire issue des pontes déposées 15 à 20 jours après l'irradiation

Les actogrammes se révèlent à nouveau positifs pour certains lots irradiés, très nettement pour le lot à 2000 rad, faiblement pour celui à 4000 rad (fig. 8). Ils demeurent nuls pour 8000 et 16 000 rad. Les radiographies montrent d'assez nombreuses galeries larvaires complètes dans le lot à 2000 rad: 22 grains attaqués plus un certain nombre de grains piqués, donnant 14 imagos. Dans le lot à 4000 rad, on ne décèle que deux grains nettement minés, mais de nombreux grains piqués; aucun imago n'est sorti de ce lot. Dans les lots à 8000 et 16 000 rad, aucune galerie larvaire n'est décelable et on ne voit que très peu de piqûres de ponte.

Les imagos issus du lot à 2000 rad donnent, en deuxième génération, une population larvaire active (fig. 8C) et il en sortit 42 imagos pour 44 galeries larvaires révélées par radiographie.

Il semble donc raisonnable de conclure de ces diverses expériences portant sur des adultes irradiés que leur fécondité diminue graduellement jusqu'à devenir nulle, du 1er jour au 15^{e} jour après l'irradiation. Mais passé ce délai, un regain de fécondité se manifeste, au moins pour les adultes irradiés à 2000 rad. Cela pourrait correspondre à une nouvelle poussée de spermatogénèse chez les mâles irradiés, comme l'avaient laissé prévoir les observations antérieures de Pesson et Vernier [8].

e) Irradiation d'adultes – Effets généraux sur la descendance

Une autre expérience a été entreprise sur des lots de 100 insectes adultes (50 mâles + 50 femelles) irradiés de 2000 à 16 000 rad, dont on a suivi la descendance pendant deux générations successives. On a noté le nombre d'imagos issus de chaque génération et le pourcentage de grains attaqués dans chaque étape de l'expérience, étant entendu que les géniteurs disposaient ici d'une masse de grain importante pour déposer leur ponte. Les résultats sont donnés à la figure 9.

On constate qu'une dose de 8000 rad est suffisante, à l'égard des adultes, pour annihiler tout développement larvaire. On remarque également que des doses d'irradiation de 2000 à 4000 rad réduisent la descendance respectivement de près de 60% et de 85%.

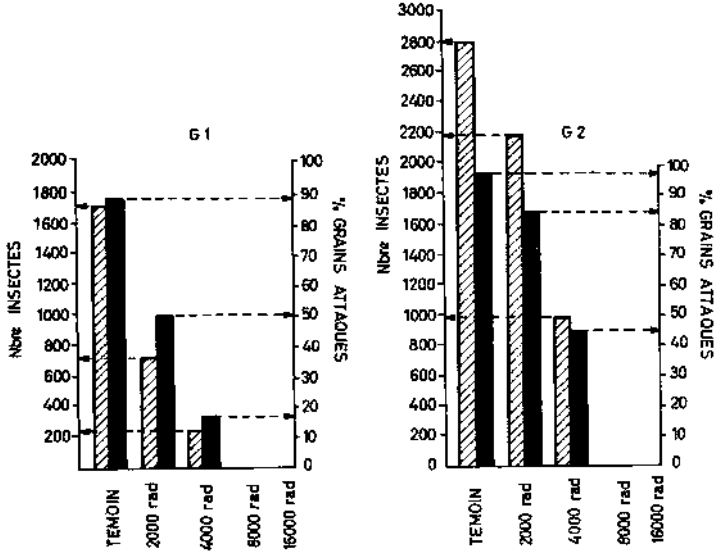

FIG. 9. Diagramme soulignant l'influence de l'irradiation sur des adultes de Sitophilus zeamais (50 couples par expérience), en comparant le nombre de leurs descendants et le nombre de grains attaqués. G1: descendance de première génération. G2 descendance de deuxième génération.

Cependant, à la deuxième génération, les adultes des lots irradiés à 2000 et 4000 rad sont capables de redonner des populations larvaires assez abondantes, qui attaquent les grains dans la proportion de 80% (2000 rad) et 40 à 45% (4000 rad).

CONCLUSION

Par l'emploi combiné de la radiographie des grains et de l'enregistrement de l'activité des stades endogés de Sitophilus zeamais à l'aide d'un détecteur électro-acoustique très sensible avec une très forte amplification, il a été possible de préciser l'effet des radiations ionisantes sur les divers stades de développement de cet insecte. La méthode permet en particulier de déceler les réductions de fertilité en mettant en évidence des piqûres de pontes non accompagnées de galeries larvaires et de stades endogés actifs (œufs stériles). Les principaux résultats observés sont les suivants:

-La sensibilité de Sitophilus zeamais aux radiations ionisantes diffère selon le stade de développement.

-Le stade « œuf » est le plus sensible, le stade « larve âgée » (19 à 21 jours) le plus résistant. Tandis qu'une dose de 2000 rad suffit pour empêcher toute éclosion des œufs, il faut 8000 rad pour arrêter complètement l'évolution des larves âgées. Cette même dose de 8000 rad, appliquée à des adultes, annihile également leur descendance larvaire.

-Une dose de 16 000 rad est nécessaire pour obtenir la destruction complète d'une population mixte de larves et d'adultes dans un lot de grains.

-Les effets des radiations à faible dose (2000, 4000 et 8000 rad) se font encore sentir sur la deuxième génération issue de larves ou d'adultes irradiés. Il s'agit en particulier d'une infertilité plus ou moins grande des œufs, conséquence vraisemblable de mutations létales dominantes, surtout fréquente dans le cas d'irradiation de larves âgées (19 à 21 jours).

-Les adultes irradiés présentent, même après des doses faibles d'irradiation (2000 rad), une diminution de leur fécondité qui va en s'aggravant du 1er au 15^{e} jour après l'irradiation, et peut à ce moment aboutir à une stérilité de fait. Mais, passé ce délai (du 15^{e} au 20^{e} jour après l'irradiation), les insectes retrouvent une certaine fécondité, conséquence vraisemblable d'une reprise de la spermatogenèse chez les mâles.

REMERCIEMENTS

Ce travail a été effectué grâce à un contrat de recherches passé avec l'INSERM (Institut national de la santé et de la recherche médicale) dans le cadre des actions concertées « Nutrition ». Il nous est agréable d'exprimer ici aux responsables de la Commission INSERM toute notre gratitude pour l'aide apportée à notre laboratoire et pour avoir ainsi facilité le développement de nos recherches sur l'irradiation des insectes des denrées.

Nous adressons également nos vifs remerciements à M. Simon, du Centre d'études nucléaires de Saclay, à M. Vallée, technicien photographe, et à Mlle Joannes, aide technique du Laboratoire de zoologie de l'Institut national agronomique, qui ont collaboré à divers titres à ce travail.

REFERENCES

[1] ADAMS, R.E., WOLFF, J.E., MILNER, M., SHELLENBERGER, J.A., Detection of internal insect infestation by sound amplification, Cereal Chem. 3 (1954) 271-76.

[2] BAILEY, S.W., McCABE, J.B., The detection of immature stages of insects within grains of wheat, J. stored Prod. Res. 1 (1965) 201-02.

[3] KIRKPATRICK, R.L., WILBUR, D.A., The development and habits of the granary weevil, Sitophilus granarius, within the kernel of wheat, J. econ. Ent. 58 3 (1965) 971-85.
[4] MILLS, R.B., WILBUR, D.A., Radiography studies of Angoumois grain moth development in wheat, corn and sorghum kernels, J. econ. Entom. 60 3 (1967) 671-77.
[5] CORNWELL, P.B., The Entomology of Radiation Disinfestation of Grains, Pergamon Press, London (1966) 236 pp.
[6] PESSON, P., Utilisation des radiations ionisantes (^{60}Co) pour la protection des denrées contre les insectes nuisibles, Recherches relatives à la détermination des doses minima utiles pour assurer la stérilité des insectes, Inds agric. aliment. 3 (1963) 211-25.
[7] PESSON, P., L'énergie nucléaire est-elle utilisable pour la protection des stocks de céréales contre les insectes?, Information et Documentation Agricoles n°2, Coopératives La Fayette, Paris (15 janvier 1965).
[8] PESSON, P., VERNIER, J.M., La protection des denrées contre les insectes ravageurs par l'emploi des radiations ionisantes en vue d'obtenir la stérilité des insectes adultes, Etude particulière de la réaction des gonades de Sitophilus granarius, Annls Nutr. et Aliment. XVII 6 (1963) 487-97.

DISCUSSION

G. LE MASNE: It would be interesting to find out from Professor Pesson or Mr. Girish whether they think the sounds recorded by the electro-acoustic detector are emitted by the larvae during feeding (by rubbing the jaws against the grain) or by moving about inside the cavity they have dug for themselves in the grain.

P. PESSON*: The vibrations recorded by the electro-acoustic detector may be due to either type of activity. The actographic recording of the entire development of a grain-weevil larva from hatching to emergence of the imago (P. Pesson and M. Ozer, Ann. des Epiphyties (1968), in the press) shows that the feeding activity of the larva is continuous, being interrupted only in the process of shedding of the larval skin. The period during which the pupal case is prepared and the pupal period itself are characterized by peculiar noises. But these peculiarities can only be detected by using the actograph on a single grain of affected corn.

W.J. KLOFT: An electro-acoustic actograph used during the irradiation process would possibly allow registration of direct responses of the insects. Do you know whether Busnel's apparatus will function when exposed to ionizing radiation? I am sure that this would provide an interesting line of experimental study.

P. PELEGRIN: I am not sufficiently well acquainted myself with Mr. Busnel's actograph to be able to reply. However, I believe it should be possible to investigate the insects during irradiation as you suggest, since the radiation doses involved are relatively weak.

* Written reply received following the Symposium.

STERILISATION DE LA PYRALE DU MAIS (Ostrinia nubilalis Hb.) PAR L'IRRADIATION AUX RAYONS GAMMA*

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MONTFAVET, FRANCE

Abstract — Résumé

STERILIZATION OF THE EUROPEAN CORN BORER Ostrinia nubilalis Hb. BY GAMMA IRRADIATION. Pupae and larvae of Ostrinia nubilalis Hb. were irradiated with a cobalt-60 source. The dose rate was about 1040 rad/min. The effects of different doses on the survival of the pupae, the length of life of the adults, sexual behaviour, fecundity and fertility were studied. Larvae at the end of their development exposed to 5, 10, 15, 20, 25 and 30 krad gave respectively 90, 77, 23, 16, 13 and 10% of pupae; the non-pupated larvae often remained alive in their cocoons for four months or more. At 10 krad and above, no adults hatched. At 5 krad, 63% of adults were obtained; the males and females which hatched showed a fertility of only about 12%, their sexual behaviour, fecundity and length of life being reduced. The sensitivity of the pupae decreased with age; 6- and 12-day-old specimens irradiated at 10 krad gave a hatching rate of 42 and 100% respectively. A dose of 20 krad applied to female 12-day-old pupae was sufficient to make the eggs infertile, while a dose of 40 krad was needed to ensure that irradiated males coupled with normal females gave rise to totally sterile batches of eggs. When groups of sterile butterflies (25 males, 25 females or 25 individuals of both sexes) were introduced into a cage containing five normal butterflies of each sex, the number of viable eggs laid was reduced, the maximum reduction being for the proportion 25 : 25 : 5 : 5. It would therefore seem advantageous to release both irradiated males and females together.

STERILISATION DE LA PYRALE DU MAIS (Ostrinia nubilalis Hb) PAR L'IRRADIATION AUX RAYONS GAMMA. Les pupes et les larves de Ostrinia nubilalis Hb. ont été irradiées avec une source au cobalt-60. Le débit de dose était environ de 1040 rads/min. Les effets de doses variées sur la survie des pupes, la longévité des adultes, le comportement sexuel, la fécondité et la fertilité ont été examinés. Des larves en fin de développement exposées à 5, 10, 15, 20, 25 et 30 krads donnent respectivement 90, 77, 23, 16, 13 et 10% de pupes; les larves non pupéfiées restent souvent vivantes dans leurs cocons pendant 4 mois ou plus. A 10 krads et plus, aucun adulte n'éclot. A 5 krads, on obtient 63% d'adultes; les mâles et femelles éclos montrent une fertilité de seulement 12% environ; mais le comportement sexuel, la fécondité et la durée de vie sont réduits. La sensibilité des pupes diminue avec l'âge; des pupes de 6 et 12 jours irradiées à 10 krads donnent 42 et 100% d'éclosions. La dose de 20 krads appliquée à des pupes de 12 jours suffit chez la femelle à rendre les œufs infertiles. Il faut une dose de 40 krads pour que les mâles irradiés, accouplés à des femelles normales, provoquent des pontes totalement stériles. Lorsque des groupes de papillons stériles (25 mâles, 25 femelles ou 25 individus de chaque sexe) sont introduits dans une cage contenant 5 papillons normaux de chaque sexe, les nombres d'œufs viables pondus sont réduits; la réduction maximale intervient pour le mélange 25 : 25 : 5 : 5. Il paraît donc plus intéressant de lâcher à la fois les mâles et les femelles irradiés.

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* Travail effectué en 1966-1967 pendant un stage en France organisé par l'ASTEF (Association pour l'organisation des stages en France).

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1. INTRODUCTION

L'action des rayons X sur la Pyrale du maïs, Ostrinia nubilalis Hb. (Lepidoptera: Pyralidæ) a été étudiée par Walker et Brindley [1]. L'action histopathologique des rayons gamma sur les tissus ovariens du même insecte a été étudiée par Sun et Lee [2].

Il nous a paru intéressant d'examiner l'action de rayons gamma d'une source de ^{60}Co sur les larves et les nymphes de la Pyrale du maïs.

2. MATERIEL ET TECHNIQUES D'ETUDE

Les insectes utilisés proviennent d'un élevage permanent d'O. nubilalis au laboratoire.

Les larves sont élevées sur un milieu artificiel à base de germe de blé suivant la méthode mise au point par Mme Guennelon, à la Station de zoologie agricole de Montfavet (travaux non publiés). Les larves sont alimentées individuellement dans des tubes en matière plastique de 5 cm de longueur et 1 cm de diamètre, remplis aux deux tiers de milieu. L'élevage est effectué dans une pièce climatisée à une température de 25°C et une humidité relative de 80%, éclairée pendant 18 heures par jour par des tubes fluorescents. Dans ces conditions, la diapause de la larve d'O. nubilalis est évitée. La nymphose s'effectue à partir du 16e jour de façon générale; la larve du dernier stade tisse un cocon de soie contre la paroi du tube avant de se chrysalider. Chaque jour les tubes contenant des nymphes sont prélevés et placés dans une autre pièce climatisée à une température de 22°C et une humidité relative de 80%. Avant l'irradiation, les nymphes sont extraites des tubes et sont séparées par sexe. La durée de la nymphose est d'environ 12 jours.

Pour l'accouplement des adultes nous avons utilisé des cages cylindriques en matière plastique de 20 cm de diamètre et 25 cm de hauteur. Les couvercles en grillage plastique sont fixés avec une bande adhésive. Pour les contrôles de fécondité des adultes et de viabilité des œufs, on tapisse les parois de la cage avec du papier sulfurisé, sur lequel les papillons déposent leurs œufs.

Une fois par semaine les adultes sont changés de cages, les pontes sont comptées. Un certain nombre de pontes sont gardées pour contrôler la viabilité des œufs, et les larves écloses sont comptées chaque jour.

On place cinq couples par cage, suivant différentes combinaisons (mâle irradié × femelle non irradiée; femelle irradiée × mâle non irradié; témoins, mâle et femelle non irradiés). On installe cinq cages par combinaison. Les femelles sont disséquées après leur mort pour compter les spermatophores.

Dans d'autres expériences comportant des mélanges de populations, des cages grillagées sont utilisées. Ces cages sont analogues à celles qu'utilisent Guthrie et al. [3]. La technique d'obtention des pontes est la même que celle adoptée par ces auteurs. Les pontes sont récoltées après 7 jours et sont gardées pour le contrôle de la viabilité des œufs.

Ces cages sont placées dans une pièce climatisée à une température de 22°C et une humidité relative de 80%, à la lumière naturelle. On utilise seulement les adultes qui ont éclos dans les 24 heures suivant l'irradiation. Les adultes sont nourris avec de l'eau sucrée à 5%.

Les irradiations ont été effectuées avec un irradiateur de ^{60}Co de 1400 Ci au Service de radio-agronomie du Centre d'études nucléaires de Cadarache. Le débit de dose était d'environ 1040 rad/min. La dosimétrie a été effectuée pour chaque exposition.

3. RESULTATS

3.1. Irradiation des larves

Les larves du dernier âge (15 j) ont été irradiées à des doses de 5 à 30 krad. Après l'irradiation les larves sont retransférées, individuellement, dans les tubes avec le milieu frais pour la nymphose.

3.1.1. Nymphoses et éclosions

Le tableau I montre que, même à la plus faible dose de 5 krad, il y a des larves qui ne se nymphosent pas; elles restent vivantes pendant plus de 4 mois dans une attitude caractéristique des larves en état de diapause (alors que les témoins dans les mêmes conditions se nymphosent très rapidement). Nous n'avons cependant pas pu obtenir de reprise de développement, bien que les larves aient été placées dans différentes conditions de température. Plusieurs auteurs [4, 5] ont déjà signalé, chez d'autres insectes, que l'irradiation des larves provoque un arrêt

TABLEAU I. EFFETS DU RAYONNEMENT GAMMA SUR LES LARVES DU DERNIER AGE D'Ostrinia nubilalis Hb.
(30 larves par dose)

Dose (krad)	Nombre de larves mortes avant la nymphose[a]	Nombre de nymphes mortes	Nombre d'adultes éclos
0	0	2	28
5	3	8	19
10	7	23	0
15	23	7	0
20	25	5	0
25	26	4	0
30	27	3	0

[a] Y compris les larves dont le développement s'est arrêté.

de développement de type diapause et Vasilyan [6] aurait montré que, chez Pectinophora malvella, l'irradiation des larves du dernier âge cause un véritable état de diapause.

Le nombre de nymphes formées devient très faible pour les doses supérieures à 10 krad. Mais aucune éclosion de nymphes n'est constatée pour des doses de plus de 5 krad appliquées aux larves du dernier âge.

3.1.2. Stérilité obtenue

Les adultes issus de larves irradiées à 5 krad montrent (tableau II) une fécondité, une fertilité et une longévité assez réduites. La fertilité est réduite à 12% environ, mais la vigueur sexuelle semble diminuée; on constate en particulier que de nombreux couples d'insectes ne peuvent pas se séparer après l'accouplement (28% des couples mâle irradié × femelle non irradiée et 16% des couples mâle non irradié × femelle irradiée).

TABLEAU II. FECONDITE, FERTILITE ET LONGEVITE DES ADULTES D'Ostrinia nubilalis Hb. ISSUS DE CROISEMENTS D'INSECTES NON IRRADIES AVEC DES INSECTES ISSUS DE LARVES IRRADIEES A 5 krad

Dose (krad)	Nature des croisements		Nombre d'œufs par femelle	Nombre d'œufs éclos	Longévité des adultes (j)	
	Mâle	Femelle			Mâle	Femelle
0	N	N	510	60,8	20,8	20,6
5	I	N	376	11,6	13,3	20,2
	N	I	76	13,7	27,3	11,6

N = non irradié, I = irradié.

3.2. Irradiation des nymphes

3.2.1. Eclosions

Des doses de 10 et 15 krad ont été appliquées à des nymphes d'âges différents. On voit dans le tableau III que ces deux doses ont à peu près la même action, l'irradiation à 12 jours ne diminuant pas les éclosions par rapport aux témoins.

Les doses de 20 à 50 krad ont été appliquées à des nymphes de 12 jours. Seule la dose de 50 krad provoque une légère diminution des éclosions par rapport aux témoins.

TABLEAU III. EFFETS DU RAYONNEMENT GAMMA SUR DES NYMPHES D'Ostrinia nubilalis Hb. A DIFFERENTS AGES (60 nymphes par cas)

Age (j)	Pourcentage d'éclosion des adultes par rapport aux témoins	
	10 krad	15 krad
Témoins	100,0	100,0
2	0,0	0,0
4	4,0	4,0
6	42,3	34,6
8	73,2	57,3
10	86,6	82,0
12	100,0	95,4

3.2.2. Stérilité obtenue

La stérilité a été examinée en mettant chaque fois en présence dans la même cage cinq mâles et cinq femelles irradiées ou non. Les doses utilisées allaient de 10 à 50 krad.

Un essai sur des nymphes âgées de 6 jours à la dose de 10 krad a montré une fertilité chez les mâles irradiés réduite à 22,7% (témoin 77,6%).

L'essai complet a porté sur des nymphes âgées de 12 jours. Le nombre moyen d'œufs pondus par des femelles normales n'est que faiblement influencé par l'accouplement avec des mâles irradiés, quelle que soit la dose. Par contre, chez les femelles irradiées, le nombre moyen d'œufs pondus diminue progressivement (fig. 1).

La fertilité des œufs pondus par des femelles irradiées croisées avec des mâles non irradiés est presque nulle dès la dose de 10 krad et devient nulle à partir de 20 krad. La fertilité des œufs résultant du croisement mâle irradié × femelle non irradiée diminue progressivement pour devenir nulle à 40 krad (fig. 2).

3.2.3. Vigueur sexuelle

La vigueur des mâles issus de nymphes irradiées à différentes doses a été examinée. Le critère était le nombre de spermatophores présents dans les femelles après leur mort. Pour des doses de 10 à 40 krad, le

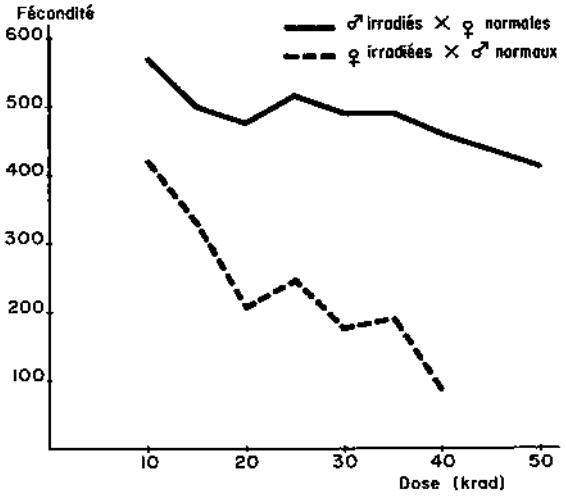

FIG. 1. Fécondité résultant de croisements insectes normaux × insectes issus de nymphes de 12 j irradiées aux doses de 10 à 50 krad (la fécondité des témoins non irradiés était de 554 œufs).

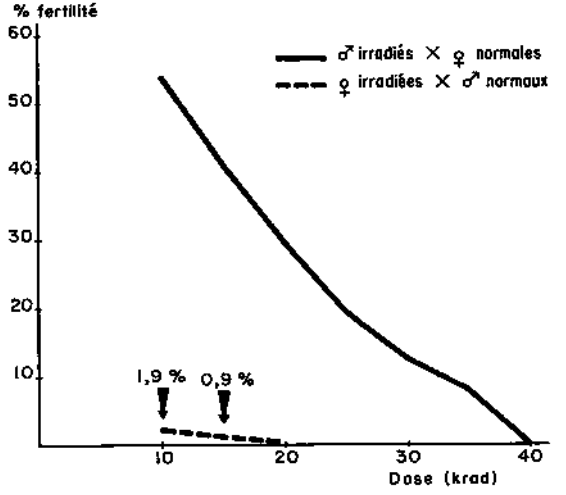

FIG. 2. Fertilité résultant de croisements insectes normaux × insectes issus de nymphes de 12 j irradiées aux doses de 10 à 50 krad (la fertilité des témoins non irradiés était de 78%).

nombre moyen d'accouplements par mâle restait comparable à celui du témoin (1,45 dans les conditions de nos expériences); il était plus réduit (0,95) pour 50 krad. La vigueur sexuelle des mâles ne paraît donc pas diminuée aux doses stérilisantes. Lorsque des femelles irradiées sont mises en présence de mâles normaux, les nombres d'accouplements par mâle ne subissent pas de diminution, ce qui montre que ces femelles restent aussi réceptives que des femelles non irradiées.

TABLEAU IV. VIABILITE DES ŒUFS D'Ostrinia nubilalis Hb. RESULTANT DU MELANGE D'INSECTES STERILES ET D'INSECTES NORMAUX EN DIFFERENTES PROPORTIONS

Nombre d'adultes par cage				Nombre d'œufs fertiles par femelle normale (première semaine de ponte)
Stériles		Normaux		
Mâle	Femelle	Mâle	Femelle	
0	0	5	5	183
0	0	5	5	204
0	0	5	5	230
0	0	5	5	218
25	25	5	5	0
25	25	5	5	0
25	25	5	5	0
25	25	5	5	52
25	0	5	5	111
25	0	5	5	14
25	0	5	5	101
25	0	5	5	54
0	25	5	5	201
0	25	5	5	102
0	25	5	5	150
0	25	5	5	26

3.3. Mélanges de populations

Quelques essais de mélanges d'insectes irradiés (à la dose de 40 krad appliquée à des nymphes de 12 jours) ou non en proportions variables ont été réalisés. Les résultats sont indiqués dans le tableau IV. Le résultat le plus remarquable ressort de la comparaison des mélanges, mâles stériles, femelles stériles, mâles normaux et femelles normales, dans les proportions: 25:0:5:5 et 25:25:5:5. Le fait de mélanger ces populations complètes, normales et irradiées, dans la proportion de 5 à 25 provoque une stérilité à peu près absolue. Par contre, si cette proportion de 5 à 25 est limitée aux mâles, la stérilité provoquée reste réduite. Ceci montre que, dans les conditions de nos essais, le fait d'ajouter des femelles stériles joue un rôle important dans la diminution de la fertilité. Le même phénomène a été mis en évidence par Husseiny et Madsen [7] sur Paramyelois transitella. Par contre, Proverbs et Newton [8], sur Carpocapsa pomonella, indiquent qu'il paraît préférable de ne pas lâcher de femelles stériles. D'autre part, dans notre essai le fait de lâcher seulement des femelles stériles (0:25:5:5) ne provoque pas régulièrement la stérilité, contrairement aux résultats obtenus par Elbadry [9] sur Gnorimoschema operculella.

Ceci montre que les résultats de mélanges de populations stériles et normales peuvent être très différents d'un insecte à l'autre. Ces différences peuvent être liées à d'importantes variations dans le comportement, surtout dans les conditions très artificielles du laboratoire. Elles ne constituent que des indications et doivent être reprises dans des conditions aussi proches que possible des conditions naturelles.

REMERCIEMENTS

L'auteur remercie Monsieur M. Féron, Directeur de la Station, qui lui a offert dans ses laboratoires de recherches toutes les facilités désirables pour mener à bien un travail scientifique, et qui a bien voulu consacrer une partie de son temps à l'étude critique de ce travail.

REFERENCES

[1] WALKER, J.R., BRINDLEY, A.T., J. econ. Ent. 56 (1963) 522-25.
[2] SUN, S., LEE, W., Acta ent. sin. 14 (1965) 423-31.
[3] GUTHRIE, W.D., RAUN, E.S., DICKE, F.F., PESHO, G.R., CARTER, S.W., Iowa St.J. Sci. 40 (1965) 65-83.
[4] BARDUGHI-BONAB, H., Etude du développement post-embryonnaire de l'ovaire chez Ephestia kühniella Z. (Lépidoptère Pyralidae). Effets des radiations ionisantes, Thèse de Docteur – ingénieur, Université de Paris (1965) 65p.
[5] PROVERBS, M.D., NEWTON, J.R., Can. J. Zool. 40 (1962) 401-20.
[6] VASILYAN, V.V., Ent. Obozr. 39 (1960) 599-604.
[7] HUSSEINY, M.M., MADSEN, H.F., Hilgardia 36 (1964) 113-37.
[8] PROVERBS, M.D., NEWTON, J.R., Can. Ent. 94 (1962) 1162-70.
[9] ELBADRY, E., J. econ. Ent. 57 (1964) 414-15.

DISCUSSION

R. CAVALLORO: Could you please give us the recipe for the synthetic medium on which you reared your O. nubilalis larvae?

M. ANWAR: Certainly; the medium used consisted of the following:

Distilled water	170 cm^3	Corn oil	0.4 cm^3
Agar agar	5 g	Wesson mineral salts	2 g
Cellulose	6 g	Brewer's yeast	6 g
Wheat germ	10 g	Ascorbic acid	1 g
Casein proteolysate	7 g	Balanced vitamins	4 g
Glucose	3.5 g	Benzoic acid	0.25 g
Cholesterol	0.6 g	Nipagin	0.20 g

R.C. VON BORSTEL: Is the species you studied here identical to that studied by Walker (Reference [1] in your paper) and do you get the same dose-effect curves for sperm irradiation?

M. ANWAR: We used the same species (Ostrinia nubilalis Hb.) but Walker's sterilizing dose was 32 krad of X-rays, administered to the adult insect, whereas ours was 40 krad of gamma radiation, administered to the mature pupae.

R.C. VON BORSTEL: I understand you used five pairs of moths in the breeding tubes in each case. It would appear that the pair matings were not wholly successful. I wonder whether one of the females in your mass matings did not mate. If such a virgin female then laid her eggs this would give an egg hatchability of 80% in the control experiment. Corresponding reductions would also occur in the irradiated groups.

M. ANWAR: We always obtained over 90% mating, in control as well as in sterile insects.

I.A. KANSU: In your work on O. nubilalis and S. exigua why did you illumine the laboratory for 18 hours a day? I always thought that these two species were less active in the light.

M. ANWAR: We used fluorescent tubes for 18 hours a day simply to prevent diapause of the larvae.

I.A. KANSU: You found a life-span of 20.8 days for the male and 20.6 days for the female of the species in this work. I was under the impression that the female lived the longer. I notice that Table II also shows a life-span of 27.3 days for the male. Could you please elaborate on these results?

M. ANWAR: My experimental work has never revealed a significant difference in longevity of the male and of the female, but I did observe that those males which mated only a few times lived longer than those which mated more often.

K.K. NAIR: Is there any evidence from your studies to support the view that a first mating is more important than subsequent matings? I raise this point because it has been shown in some species that if a normal female is mated to an irradiated male and then mated to a normal male most of the eggs are found to be sterile.

M. ANWAR: I am afraid I have not studied this point.

B.R. LEROI: For your experiment on mixing together normal and sterile males and females (Table IV) have you any results for after the first week of oviposition? I am trying to ascertain whether when normal

females show no indication of fertility this is an inherent incapability or whether it is simply due to a time-lag in the physiological process involved.

M. ANWAR: No, I observed this insect for a period of one week only. The female moths laid most of their eggs within the first week after being put in the cages.

C.A. PELERENTS: Do the virgin females oviposit and are there any features whereby it is possible to distinguish between the sexes at the larval and pupal stages?

M. ANWAR: Our results indicated that virgin females could lay up to 429 eggs. We did not differentiate the sexes at the larval stage, before irradiation, but this was done on the pupae by examining the underside of the abdomen with a magnifying glass. In this case, as for many species of Lepidoptera, the position and appearance of the genital orifice is different for the male and female.

B. NAGY: Could you give any more details as to the stage of development of those embryos which died in the egg? I should be interested to learn whether they died in an early or late stage of their embryonic life.

M. ANWAR: We irradiated the male moths at the pupal stage with sterilizing doses of up to 40 krad, and found that there was embryonic development up to the 'black head' stage in about 90% of the eggs laid by the females mated with them. However, these larvae could not hatch out of the shell and eventually died.

B. NAGY: Do you think it will be feasible to control the European corn borer by means of the sterile-male technique? One has to bear in mind that this species is very widely distributed and the adults can fly many miles in one night during the flight stage, especially in stormy, windy weather and this may contribute significantly to dispersal of the adults.

M. ANWAR: My laboratory results, set out in Table IV, showed clearly that sterile insects are competitive with normal insects. It is quite probable that they will compete in the field as well.

V. LABEYRIE: The second set of results in Table IV of your paper shows only one group of females out of the four as laying eggs. Does this mean that the five females of each group remained virgins and hence that the normal males must have selected the sterile females, or that the females mated with sterile males? Did you investigate for spermatophores inside the females?

M. ANWAR: The adults of O. nubilalis are capable of mating from the first two days after hatching. I studied these insects for seven days and I do not think that the females remained unmated for as long as that. I did not, however, dissect the females to look for spermatophores.

INFLUENCE OF DIAPAUSE ON THE RADIOSENSITIVITY OF KHAPRA BEETLE LARVAE

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Abstract

INFLUENCE OF DIAPAUSE ON THE RADIOSENSITIVITY OF KHAPRA BEETLE LARVAE. Studies to determine the effects of varying periods of diapause on the radiosensitivity of the larvae of Trogoderma granarium showed practically no mortality in the irradiated larvae during diapause, but only after the diapause was broken at 38°C. As the calculated LD_{50}s for 10, 20 and 30 days of post-irradiation diapause were not significantly different, it was also evident that increases in the length of diapause time had no significant effect on post-diapause survival time. On the other hand, pupation seemed to be influenced by the duration of diapause in the irradiated larvae. This effect was best discernible at low doses. The significance of these findings is discussed.

1. INTRODUCTION

In a previous communication [1], the authors reported that when the larvae of the Khapra beetle, Trogoderma granarium Everts, were irradiated with 5 and 8 krad and maintained in a diapausing state, mortality was remarkably low, and that it manifested itself after the diapause was broken by raising the ambient temperature to 38°C. Further studies were conducted with the same species to determine whether increases in diapause time at a much lower temperature (20°C), after irradiation with various doses of gamma radiation, would influence its radiation sensitivity with respect to larval mortality and pupation.

2. MATERIALS AND METHODS

The larvae of the Khapra beetle used in these studies were collected at random from a laboratory culture of 5th and 6th instar larvae maintained at 30° ± 2 degC. They were irradiated with doses ranging from 5 to 18 krad in a cobalt-60 source at a dose rate of 1.5×10^5 krad/h. After irradiation the larvae were separated into three groups and kept at 20° ± 0.5 degC with crushed wheat as food. After 10, 20 and 30 days at this temperature the diapause was broken by transferring the larvae to 38° ± 1 degC and they were maintained at this temperature till the end of the experiment. Four replicates of 50 larvae each were used for each irradiation dose level. The same number of replicates of 50 larvae each were simultaneously kept as control. Larval mortality and pupation in

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the various groups were recorded at daily intervals. The data on the cumulative mortality after 10 days of post diapause were subjected to probit analysis for the calculation of LD_{50}. The larval mortality that occurred during diapause was not considered in calculating LD_{50}.

3. RESULTS AND DISCUSSION

It is evident from the results that practically no mortality occurred in the irradiated larvae during diapause at 20°C (Figs 1-3). Radiation damage manifested itself only after the diapause was broken at 38°C. The calculated LD_{50} values for 10, 20 and 30 days of diapause were 16.22, 16.03 and 16.9 krad, respectively. As these values did not differ significantly, it was also evident that increases in the length of diapause time had no significant effect on post-diapause survival time (Table I).

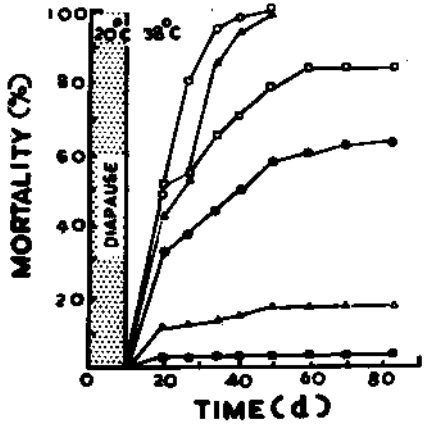

FIG. 1. Effect of 10 days' post-irradiation diapause on larval mortality
■ = 5 krad; Δ = 6 krad; ● = 8 krad; □ = 10 krad; ▲ = 14 krad; ○ = 18 krad

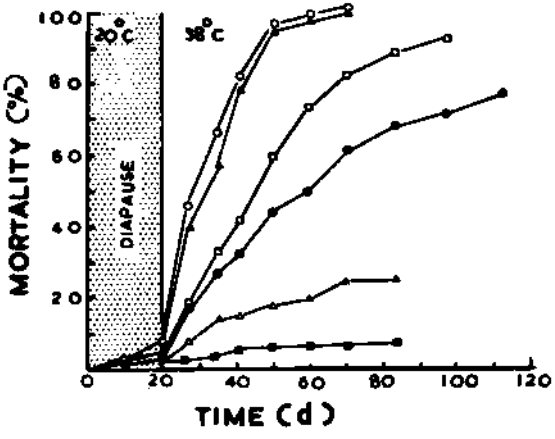

FIG. 2. Effect of 20 days' post-irradiation diapause on larval mortality

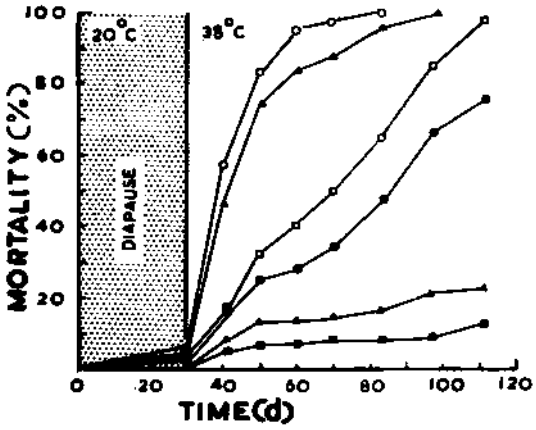

FIG. 3. Effect of 30 days' post-irradiation diapause on larval mortality

TABLE I. EFFECT OF DURATION OF POST-IRRADIATION DIAPAUSE ON THE LD_{50} (10 DAYS) FOR THE LARVAE OF Trogoderma granarium EVERTS

Post-irradiation diapause in days	Heterogeneity	Regression equation	LD_{50} (krad) ± S.E.	Fiducial limits of LD_{50} with 95% confidence
10	$\chi^2(5) = 28.1$	$Y = 2.019 + 2.464\ X$	16.22 ± 0.06	15.080
				17.360
20	$\chi^2(5) = 7.2$	$Y = 1.469 + 2.93\ X$	16.03 ± 0.02	14.935
				17.125
30	$\chi^2(5) = 15.0$	$Y = 0.489 + 2.676\ X$	16.9 ± 0.017	15.896
				17.004

Y = Probit kill; X = Lg dose; LD_{50} = Radiation dose calculated to give 50% kill.

Burges [2] studied the behaviour of diapausing and non-diapausing larvae of Trogoderma granarium. He observed that a dormant larva spent long periods without feeding or moving and that its rate of respiration was probably at a basal level. However, when the temperature became favourable, the larva emerged from the dormant state, with a consequent increase in its rate of respiration, which was about two to six times that of a dormant larva. The inverse relationship between survival of an irradiated organism and its metabolic rate is well known [3]. In the diapausing state, radiation damage remains latent and expresses itself only when the metabolic rate is accelerated by breaking the diapause. This explains the absence of any protective effect by the intervening

diapause. A similar effect was also observed in hibernating squirrels [4] and marmots [5].

Pupation occurred only after the diapause was broken and occurred mostly within the first 10 days of post-diapause period. Although it was observed at doses of 10 krad and below, adult emergence was seen only up to 6 krad, indicating that at 8 and 10 krad death occurred in the pupal stage (Table II).

TABLE II. EFFECT OF POST-IRRADIATION DIAPAUSE ON PERCENTAGES OF ADULT EMERGENCE (Trogoderma granarium) FROM PUPAE FORMED

Dose (krad)	Post- irradiation diapause (days)		
	10	20	30
0	96.3	98.9	99.5
5	83.7	85.5	88.3
6	56.6	63.3	58.2
8	0.0	0.0	0.0
10	0.0	0.0	0.0

The duration of diapause alone had no significant effect on the number pupated in the non-irradiated control. Pupation was 94.5, 94.0 and 92.0%, respectively, when the diapause lasted for 10, 20 and 30 days. However, pupation in the irradiated groups was influenced, not only by the radiation dose, but also by the duration of post-irradiation diapause (Fig. 4). Analysis of variance of the data on pupation in the irradiated groups subjected to different post-irradiation diapause periods showed that the differences observed in pupation were significant at 1% level. The F value for interaction between radiation and diapause is 6.17**. It was observed that the number pupating in the irradiated groups tended to decrease with increases in diapause time. This suggested increased radiation damage in those subjected to the longer post-irradiation diapause. In our earlier studies (loc. cit.) it was observed that when the irradiated larvae were maintained continuously at 38°C, pupation occurred up to a dose of 12 krad, while in the present studies it occurred only up to a dose of 10 krad when a post-irradiation diapause intervened. These results suggested a more rapid progression of radiation damage in the larvae subjected to diapause at 20°C than in those maintained without a diapause. The factor that contributed to this acceleration of damage could be due to the higher metabolic rate of the larvae preconditioned to a temperature of 20°C. The rates of oxygen consumption of the larvae reared at 38°C and those of the larvae in which diapause was induced at 20°C for 10 days were determined in a Warburg apparatus at 38°C. The oxygen consumption of the former was 160 μl/mg per hour,

** = very significant

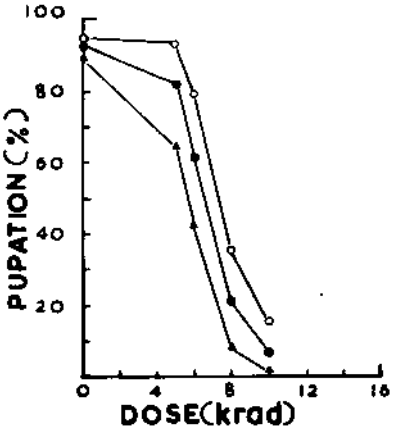

FIG. 4. Effect of 10 (o), 20 (•) and 30 (▲) days' post-irradiation diapause on pupation

while that of the latter was 203 μl/mg per hour. This difference was statistically significant ($P = 0.01$). The increased rate of oxygen consumption indicated increased metabolic rate which would then result in a more rapid progression of the damage in the irradiated larvae. This could account for the absence of pupation above 10-krad level when a post-irradiation diapause intervened. The same metabolic effect could also explain the damage differential observed when the post-irradiation diapause time was increased. Williams [6] studied the effects of graded exposures of the diapausing pupae of Cecropia silkworm to 6°C on the initiation of adult development after transferring them to 25°C. He observed that for 5, 10 and 20 weeks of diapause the rate of development increased markedly with the length of diapause time. This suggested an accelerated metabolic rate according to the length of prior chilling. Though the authors did not determine the oxygen consumption of the Khapra beetle larvae subjected to diapause for 20 and 30 days, their results for the 10-day diapause period and those of Williams (loc. cit.) showed that the metabolic rate was considerably influenced by the duration of diapause in the cold. Larvae which had a longer period of diapause showed a higher metabolic rate when the diapause was broken. As the time of survival of an irradiated organism is inversely proportional to the metabolic rate, the radiation damage progressed more rapidly in the larvae subjected to longer post-irradiation diapause than in those exposed for a shorter period. This explained the progressive decrease in pupation according to the increase in the length of the post-irradiation diapause.

Since high doses of radiation tended to limit pupation, the influence of post-irradiation diapause was best discernible at lower doses. At 10 krad this effect appeared more or less masked by the effect of the radiation dose itself. This, apparently, was the reason why there was no influence of the post-irradiation diapause on the LD_{50} for larval mortality, as no pupation occurred at this dose level (16 krad).

REFERENCES

[1] NAIR, K.K., RAHALKAR, G.W., in Radiation and Radioisotopes applied to insects of Agricultural importance, IAEA, Vienna (1963) 465-77.
[2] BURGES, H.D., J. Insect Physiol. 5 (1960) 317.
[3] BACQ, Z.M., ALEXANDER, P., Fundamentals of Radiobiology, Pergamon Press, New York (1961) 3.
[4] DOULL, J., DUBOIS, K.P., Proc. Soc. exp. Biol. Med. 84 (1953) 367.
[5] SMITH, F., GRENAN, M.M., Science 113 (1951) 686.
[6] WILLIAMS, C.M., Biol. Bull. 110 (1956) 201.

DISCUSSION

H. ERDMAN: Have you tried modifying the gaseous environment, for instance by using nitrogen instead of air, during radiation exposure and afterwards in order to determine the effect of different atmospheres on diapause and radiosensitivity?

G. W. RAHALKAR: No, we have not.

I. A. KANSU: Did you observe any diapause at the early larval stage in the Khapra beetle?

G. W. RAHALKAR: Diapause in this insect is influenced by environmental factors and is not peculiar to any particular developmental stage.

EFFETS DES RADIATIONS GAMMA SUR LA FERTILITE ET LA LONGEVITE DES COLONIES DE Dolichoderus quadripunctatus (HYMENOPTERE: FORMICOIDEA DOLICHODERIDAE)

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Abstract — Résumé

THE EFFECT OF GAMMA RADIATION ON THE FERTILITY AND LONGEVITY OF Dolichoderus quadripunctatus (HYMENOPTERA: FORMICOIDEA DOLICHODERIDAE). The authors studied on a batch of 27 colonies of the ant Dolichoderus quadripunctatus the effect on longevity and fertility of gamma irradiation at doses between 12 500 and 150 000 rad. The results are presented in the paper. For colonies consisting solely of workers, the mortality curve is linear with doses between 150 000 and 50 000 rad. The LD_{50} is reached in 4 days for the 150 000-rad dose and 16 days for the 50 000-rad dose. The lowest dose used in the experiments (12 500 rad) produced an LD_{50} after 18 days. For the control colonies, the LD_{50} occurred within about thirty days.

Fertility, which is normally regular and constant in colonies of workers of Dolichoderus quadripunctatus is expressed by the R.F. ratio [R.F. = reproduction factor = (number of eggs)/(number of workers at the beginning of the experiment)]. The R.F. of the control (unirradiated) colonies was about 1.60, while that of the irradiated colonies ranged from 0 to 0.06. These extremely low values showed that to all intents and purposes the treated colonies were made sterile. This sterility was final, no laying being observed in the irradiated colonies, by the time they died out.

In colonies with a queen, only the queens were irradiated, with gamma doses of 50 000, 100 000 and 150 000 rad. The results obtained for longevity showed in every case a considerably higher resistance of the females than the workers under the same conditions. Where fertility is concerned, the irradiated females showed a considerable and almost immediate reduction in egg laying. This was followed in the colonies by a resumption of laying by the workers, since the queen could no longer inhibit this. The fertility of the irradiated females subsequently diminished, and became nil from the eighteenth day. The queens did not thereafter resume laying.

EFFETS DES RADIATIONS GAMMA SUR LA FERTILITE ET LA LONGEVITE DES COLONIES DE Dolichoderus quadripunctatus (HYMENOPTERE: FORMICOIDEA DOLICHODERIDAE). Les auteurs on étudié sur un lot de 27 colonies (avec ou sans reine) de la fourmi Dolichoderus quadripunctatus les effets, sur la longévité et la fertilité, d'irradiations gamma à des valeurs comprises entre 12 500 et 150 000 rad; les résultats obtenus sont donnés dans le mémoire.

Dans le cas des colonies exclusivement composées d'ouvrières la courbe de mortalité est linéaire pour des valeurs comprises entre 150 000 et 50 000 rad. La DL 50 est atteinte en 4 jours pour la dose de 150 000 rad, et 16 jours pour la dose de 50 000 rad. Aux valeurs les plus faibles (12 500 rad) la DL 50 s'obtient après 18 jours. Pour les colonies témoins la DL 50 s'obtient en 30 jours environ. La fécondité, qui est habituellement régulière et constante dans les colonies d'ouvrières, est exprimée par le rapport QR = quotient reproducteur = (nombre d'œufs/nombre d'ouvrières au début de l'expérience). Le QR des colonies témoins (n'ayant subi aucune irradiation) a pour valeur 1,60 environ. Le QR des colonies traitées varie entre 0 et 0,06. Ces valeurs extrêmement faibles montrent que les colonies traitées sont devenues

pratiquement stériles. Cette stérilité est définitive, car jusqu'à l'extinction de la colonie, aucune ponte n'a pu être observée dans les colonies irradiées.

Dans les colonies avec reine, seules les reines subissent une irradiation gamma, aux valeurs de 50 000, 100 000 et 150 000 rad. Les résultats obtenus concernant la longévité montrent dans tous les cas une résistance considérablement plus élevée des femelles que pour les ouvrières placées dans les mêmes conditions. Pour ce qui est de la fécondité, on observe chez les reines irradiées un ralentissement considérable et quasi immédiat de leur ponte. Ce ralentissement est suivi dans les colonies par une reprise de la ponte des ouvrières: la reine ne peut plus exercer l'inhibition de leur ponte. Par la suite, la fécondité des reines irradiées diminue et devient nulle à partir du 18^{e} jour. Il n'y a plus de reprise de la ponte des reines par la suite.

INTRODUCTION

Dolichoderus quadripunctatus est une fourmi arboricole que l'on rencontre couramment dans les forêts de chêne de la région Toulousaine. Elle constitue des colonies polycaliques. On rencontre essentiellement deux types de colonies: les colonies exclusivement composées par des ouvrières, et les colonies composées par des ouvrières accompagnées d'une reine [1, 2].

Lorsqu'elles évoluent sans relations entres elles, les colonies du premier type (colonies d'ouvrières) pondent abondamment, et les œufs donnent naissance à des mâles. Les colonies du second type (colonies d'ouvrières avec une reine) se caractérisent au contraire par une ponte exclusive de la reine. Les ouvrières en présence de la reine ne pondent jamais [1, 3, 4]. Les œufs de reine donnent naissance, soit à des ouvrières (cas général), soit à d'autres reines (dans des conditions particulières que nous avons définies et exposées dans de précédents mémoires [5]).

Le présent travail est consacré à l'étude de l'action des radiations gamma sur ces deux types de colonies.

Conditions de l'expérience

Vingt-sept colonies constituées par des populations d'ouvrières comprises entre 107 et 302 ouvrières, avec et sans reine, sont récoltées au sortir de l'hibernation naturelle. Un premier lot T de neuf colonies (six avec reine, trois sans reines) constitue le lot témoin.

Un deuxième lot de 18 colonies, dont 12 exclusivement composées d'ouvrières, constitue le lot traité.

Les doses de rayonnement gamma reçues par les colonies d'ouvrières sont de 12 500, 25 000, 50 000, 75 000, 100 000 et 150 000 rad.

Les reines du lot traité (des colonies avec femelle), reçoivent respectivement 50 000, 75 000 et 100 000 rad.

Les irradiations ont été effectuées avec un irradiateur au ^{60}Co de 2 000 Ci, au Service de radioagronomie du CEN de Cadarache. Le débit de dose a été de 1 040 rad/min, contrôlé par une dosimétrie au sulfate ferreux.

1. COLONIES EXCLUSIVEMENT COMPOSEES D'OUVRIERES

1.1. Etude de la mortalité

Le tableau I récapitule les résultats que nous avons obtenus. Nous avons considéré la dose létale 100% (DL 100, temps à partir duquel toutes

les ouvrières de la colonie sont mortes). Ensuite, à partir de ce tableau, nous avons tracé les courbes des figures 1 et 2, qui représentent graphiquement les résultats précédents. La figure 1 exprime la mortalité (DL 50) en fonction de la dose de rayonnement gamma reçue en une seule exposition. La figure 2 représente la mortalité des colonies d'ouvrières en considérant la DL 50 et la DL 100.

Discussion

La figure 1 montre une courbe pratiquement linéaire entre les points B et C. La mortalité est donc exactement proportionnelle à la dose de rayonnement reçue entre les valeurs de 50 000 rad et 150 000 rad. Au-dessous de ces valeurs, c'est-à-dire de 12 500 rad à 50 000 rad (partie AB de la courbe), la mortalité paraît proportionnellement plus importante lorsque les doses décroissent. (Une série d'expériences pour les valeurs inférieures à 12 500 rad est en cours d'étude.)

La figure 2 est tracée pour étudier les variations possibles de la mortalité entre le début et la fin de l'expérience. Nous avons considéré le rapport (DL 100 - DL 50)/DL 50. Tous les points du graphique étant situés au-dessus de la ligne pointillée marquant le rapport 1, on peut en conclure que la longévité est proportionellement plus longue lorsque la première moitié de la population a disparu.

1.2. Etude de la fécondité

Il est bien connu, à la suite des travaux de divers auteurs, notamment Cole et al. [6], Narayanan et al. [7], Drummond [8] et Hennebery [9], que, outre ses effets sur la longévité, l'irradiation gamma, à des doses variables selon les espèces, modifie profondément la fécondité des individus irradiés, et la supprime totalement au-delà de certaines valeurs.

Nous avons élevé les colonies précédentes et avons, dans le même temps, étudié leur fécondité en dénombrant les œufs pondus par les ouvrières irradiées et en suivant leur devenir.

Pour exprimer plus commodément la fécondité d'une colonie nous convenons d'étudier le rapport QR, c'est-à-dire le quotient reproducteur (QR = nombre d'œufs pondus/nombre d'ouvrières présentes au début de l'expérience). Les résultats que nous avons obtenus figurent dans le tableau II.

Discussion

Etude du QR. L'examen du tableau II fait apparaître pour les témoins un QR normal c'est-à-dire un QR dont la valeur est sensiblement voisine de 1,6. Par contre, les traitements imposés déterminent une stérilité quasi totale aux doses de 12 500, 25 000, 50 000 et 75 000 rad, et totale pour les doses plus élevées de 100 000 et 150 000 rad. (Les très faibles valeurs du QR pour les doses comprises entre 12 500 et 75 000 rad ne permettent même pas une représentation graphique, si on les compare au QR des témoins.) Cette faible fécondité «résiduelle» peut s'expliquer si l'on considère que ces doses de rayonnement gamma permettent une vie relativement beaucoup plus longue que les irradiations de 100 000 et 150 000 rad.

TABLEAU I. MORTALITE DES COLONIES D'OUVRIERES

Dose de rayonnement (rad) / Mortalité (j)	12 500		25 000		50 000		75 000		100 000		150 000		Témoins		
Colonie	1	2	3	4	5	6	7	8	9	10	11	12	T1	T2	T3
DL 50	18,50	17,20	16	18,5	15,40	16	12,50	13,25	9	9	4,50	3,75	30	29,5	28,5
DL 100	48	41	39	44	38	45	25	29	24	20	9	9	81	81	74
$\frac{DL\,100 - DL\,50}{DL\,50}$	1,59	1,38	1,06	1,38	1,47	1,81	1,00	1,18	1,66	1,22	1,00	1,40	1,70	1,74	1,60
DL 50 moyennes	17,85		17,25		15,70		12,87		9		4,12		29,33		
DL 100 moyennes	44,5		41,50		41,50		27		22		9		78,66		

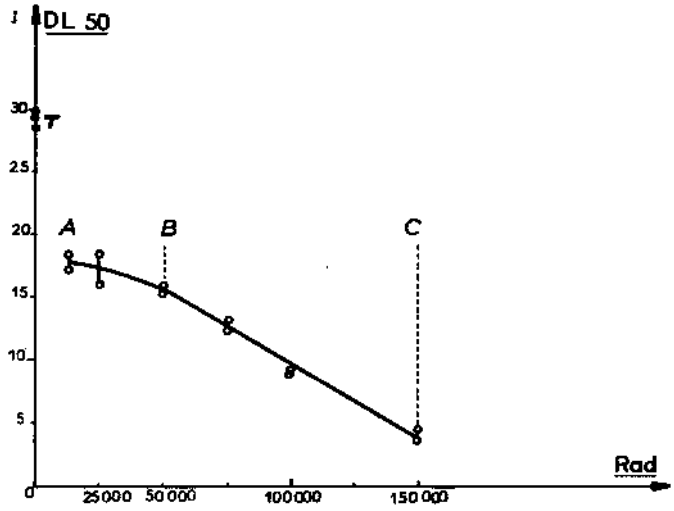

FIG. 1. Courbe de la mortalité en fonction de la dose de rayonnement gamma reçue en une seule exposition. Le point T représente la DL50 des témoins.

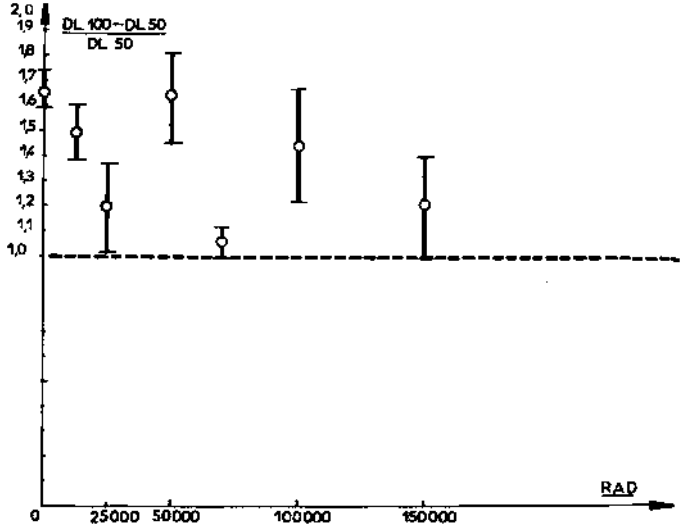

FIG. 2. Mortalité des ouvrières irradiées.

Temps pendant lequel les œufs existent dans la colonie. L'étude de ce critère n'est pas significative. Les quelques rares œufs pondus par les individus irradiés restent dans la colonie un temps comparable à celui des témoins.

Devenir du couvain. Les œufs issus d'individus irradiés ne sont pas susceptibles (généralement) d'un développement normal. Habituellement, de tels œufs présentent un aspect fripé et plissé qui contraste avec l'aspect

TABLEAU II. PRINCIPAUX RESULTATS OBTENUS DANS L'ETUDE DE LA FECONDITE DES OUVRIERES

Dose de rayonnement gamma (rad)	Colonie	Nombre d'œufs	QR	Longueur de la ponte (j)	Devenir du couvain	QR moyen
12 500	1	3	0,0280	24	néant	0,022
	2	2	0,0172	9	néant	
25 000	3	5	0,0250	16	néant	0,030
	4	6	0,0348	18	néant	
50 000	5	6	0,0352	9	néant	0,018
	6	0				
75 000	7	0				0,030
	8	7	0,0614	12	2 jeunes larves	
100 000	9	0				
	10	0				
150 000	11	0				
	12	0				
Témoins	T1	210	1,2426	23	nombreux mâles	1,592
	T2	190	1,5200	34	nombreux mâles	
	T3	260	2,0155	31	nombreux mâles	

d'un œuf normalement embryonné. Exceptionnellement, dans une seule colonie, les deux œufs pondus ont donné naissance à deux larvules du premier stade, qui n'ont cependant jamais évolué au-delà de ce stade et ont fini par disparaître de la colonie après quelques jours. Dans le même temps, les œufs des colonies témoins ont donné naissance, suivant le processus habituel déjà décrit [3] à de nombreux mâles (de 50 à 80 par colonie). Il semble donc que l'irradiation gamma à partir de 12 500 rad stérilise totalement les ouvrières. Si par hasard, semble-t-il, quelques rares œufs ou très rares larves réussissent à apparaître, ils ne sont pas viables. En outre, cette stérilité est définitive, car jusqu'à l'extinction totale des colonies irradiées, aucune reprise des pontes n'a jamais pu être observée.

2. COLONIES AVEC REINE

Un lot de 12 colonies récoltées en un seul prélèvement dans un même biotope est utilisé pour cette expérience. Six colonies constituent le lot témoin. Les six colonies traitées reçoivent les doses suivantes: deux reines (première expérience) reçoivent 50 000 rad, deux reines (deuxième expérience) reçoivent 75 000 rad, deux reines (troisième expérience) reçoivent 100 000 rad.

Remarque. Cette expérience constitue une expérience préliminaire, dont les résultats sont donnés ici à titre purement indicatif (pour être comparés à ceux des expériences précédentes intéressant les colonies d'ouvrières). Une expérimentation plus poussée est en cours actuellement, dont les résultats feront éventuellement l'objet de communications ultérieures.

2.1. Etude de la mortalité

Les reines ayant reçu 50 000 rad sont mortes après 112 jours d'élevage. Les reines ayant reçu 75 000 rad sont mortes après 35 et 32 jours d'élevage (soit en moyenne 33, 5 jours). Les reines ayant reçu 100 000 rad sont mortes après 35 et 26 jours d'élevage (soit en moyenne 30, 5 jours). Les reines des colonies témoins (n'ayant reçu aucune irradiation gamma) sont toujours vivantes après 172 jours d'élevage. Rappelons, à titre indicatif, que les ouvrières ayant reçu les mêmes doses d'irradiation (50 000, 75 000, et 100 000 rad) avaient une DL 100 de 41, 5, 27 et 22 jours, et de 78, 7 jours pour les témoins.

Discussion

Ces résultats paraissent indiquer, en valeur absolue, une résistance bien plus grande des reines comparée à la résistance des ouvrières. On sait néanmoins que les reines fécondées sont de loin les individus les plus résistants de la colonie, et ceux qui ont la plus longue vie imaginale. Il est donc possible que la résistance plus élevée des reines aux radiations gamma soit une des manifestations de cette résistance générale plus élevée.

2.2. Etude de la fécondité

L'étude de la fécondité des reines irradiées nous a permis de réaliser expérimentalement la levée de l'inhibition de la ponte des ouvrières en présence de leur reine [10].

D'une façon générale, les reines irradiées subissent des perturbations profondes dans leur rythme de ponte. Nous avons vérifié au moyen de méthodes biométriques [10] que, pour des traitements de 50 000 et 75 000 rad, la ponte des reines diminue considérablement, puis reprend légèrement, pour finalement s'arrêter définitivement à partir du 18e jour. Il n'y a plus de reprise de la ponte des reines par la suite.

Parallèlement, 3 à 4 jours après l'irradiation de la reine, les ouvrières commencent à pondre. La ponte s'effectue dans le voisinage immédiat de la reine. Il convient de remarquer cependant que, exception faite de son rythme de ponte modifié, la reine irradiée a un comportement absolument identique à celui de la reine n'ayant subi aucune irradiation.

En particulier, elle se tient en permanence sur le tas d'œufs et de larves, et ses relations trophallactiques avec les ouvrières sont normales.

La ponte des ouvrières augmente par la suite, au fur et à mesure que celle de la reine diminue.

Discussion

Devenir des œufs dans une colonie dont la reine a subi une irradiation gamma. Après irradiation, les œufs de reine présents dans la colonie, identifiables avec certitude par la méthode biométrique, présentent quelques jours après la ponte un aspect inhabituel. Les œufs paraissent «fripés». Le chorion est plissé, affaissé par endroits. Après 10 à 15 jours les œufs sont fortement ratatinés. Aucun de ces œufs n'est viable. Les œufs pondus par les ouvrières (individus non irradiés) présentent l'aspect habituel d'œufs embryonnés. Ils donnent naissance à l'habituelle génération de mâle.

CONCLUSIONS

Ces expériences révèlent donc une excellente résistance des fourmis de l'espèce Dolichoderus quadripunctatus au rayonnement gamma, ainsi que le prouve la DL 100 pour des taux d'irradiation inférieurs ou égaux à 50 000 rad. A ces doses de radiations déjà très élevées, la résistance des individus adultes est de l'ordre de 42 à 43 jours pour les ouvrières et 112 jours pour les reines. La survie des individus ainsi irradiés est donc sensiblement égale à la moitié de celle des témoins.

La fécondité apparaît par contre affectée de façon beaucoup plus profonde: une dose de rayonnement gamma de l'ordre de 12 500 rad stérilise complètement les ouvrières pondeuses.

REFERENCES

[1] TOROSSIAN, C., Recherches sur la biologie et l'éthologie de Dolichoderus quadripunctatus (L)-Hym. Formicoïdea Dolichoderidæ, Doctorat d'Etat de sciences naturelles, Toulouse (1966-1967) 1-280.
[2] TOROSSIAN, C., Insectes soc. (sous presse).
[3] TOROSSIAN, C., Insectes soc. VII (1960) 383.
[4] TOROSSIAN, C., Insectes soc. (à paraître, 1967).
[5] TOROSSIAN, C., C. r. Séanc. Soc. Biol. (sous presse).
[6] COLE, M. M., LA BRECQUE, G.C., BURDEN, G. S., J. econ. Ent. 52 (1959) 448.
[7] NARAYANAN, E.S., CHAWLA, S.S., GHAI, S., Proc. nain. Inst. Sci. India B 29 (1963) 613.
[8] DRUMMOND, R.O., Int. J. Radiat. Biol. 7 (1963) 491.
[9] HENNEBERY, T.J., J. econ. Ent. 3 (1963) 279.
[10] TOROSSIAN, C., CAUSSE, R., C. r. hebd. Séanc. Acad. sci. 264 (1967) 2789.

DISCUSSION

W. J. KLOFT: I want to extend my congratulations to you on your results, which were especially interesting from the point of view of social organization in ants. I was most struck by the way in which you achieved ovarial inhibition — up to total sterilization — of the queen without any

disturbance of the pattern of behaviour of the rest of the colony. The danger of disruptive side-effects is always present when chemosterilants are used with social insects because of regurgitation of the substances. You mentioned that the workers start to lay eggs, but that the queen nevertheless continues to play the dominant role indicative of normal behaviour. If this can be taken as a definitive result it would indicate the existence of certain 'queen-substances' (pheromones), one of them linked with fertility of the workers and the other with the social role of the queen, perhaps in the form of some attractive substance. It would be interesting to carry out similar experiments with ants of other sub-families.

C. TOROSSIAN: Thank you for your interest; I shall try to expand a little on my subject. Work on the effect of gamma rays on ants of the species Dolichoderus quadripunctatus was initially undertaken with the object of investigating the queen-worker relationship in respect of egg-laying within the colony. I feel I may confidently elaborate on the conclusions reached in my paper, particularly as regards the tendency to oviposition in the worker ants.

By suitable irradiation of the queen we succeeded in inducing rapid sterilization without loss of vitality; a few days after irradiation the queen ceases laying but her behaviour apparently undergoes no other modification. In particular, she takes up a permanent position on the pile of eggs and young larvae produced largely by the female workers. She is fed as normal by the workers by stomodeal and proctodeal trophallactic exchanges typical of this species (see [1 - 3]).

At the same time it is found that the workers lay eggs immediately on coming into contact with the queen (I have even photographed this behaviour). This result was never achieved by any previous sterilizing method. In fact, this technique of irradiating the queen of a colony of insects is rather interesting, since it is apparently the only one known to date allowing selective sterilization of the queen or of a selected group of individuals. Provided that the gamma-ray dosage is chosen carefully the individual irradiated can be sterilized without appreciable effect on its life and behaviour pattern.

It would appear from past and present work on D. quadripunctatus that inhibition of oviposition in the workers in the presence of their queen was the combined result of several sense stimuli (olfactory, visual, tactile; see [1]) and in particular those provided by chemical substances secreted by the queen and the presence of eggs laid as usual by the queen in the nest.

Lastly, I would like to mention that work is still going on and will be continued with the aim of studying the response of D. quadripunctatus to small doses of radiation and the reactions of the queens of different species of ants and other social insects to gamma irradiation.

J. H. G. TICHELER: Do you consider that the workers are induced to lay eggs after irradiation of the queen by virtue of the fact that she ceases laying herself or because she stops secreting certain chemical substances, such as pheromones?

C. TOROSSIAN: I tried for several years to cause total inhibition of oviposition in the workers by using royal 'extracts' from Dolichoderus queens (this work forms part of my Doctoral Thesis [1]) but was never successful.

In this case, after suitable irradiation the queen was completely sterilized, whereupon (i.e. 3 to 4 days later) the workers of the colony, while still behaving quite normally towards her, began to lay eggs. It would thus seem conclusive that the missing factor, absence of which induces egg-laying in the queen, is the interruption in normal accumulation of 'extracts' produced by her.

This naturally leads one to think that the presence of royal 'extract' within the group of workers is an important factor, possibly a determining one, in causing inhibition of egg-laying by female workers.

G. LE MASNE: What are the relative lengths of the irradiation period and the period of separation of the queen from her colony? It would seem important that these should be as close as possible, since undue extension of the separation period would probably have an effect on the queen-worker relationship.

C. TOROSSIAN: Irradiation time varied from a few minutes to about three hours for the largest dose. As soon as the irradiation treatment was over the queen was put back into the colony, and it appeared that the queen-worker relationship was in no way affected by this separation, which is inevitably entailed by the irradiation process. In the most interesting case (50 000 rad), the process lasted about 48 minutes; but over a separation of 24 hours did not change the queen-worker behaviour pattern. We have confirmed this many times.

G. LE MASNE: Have you investigated the ovaries of irradiated queens? Does irradiation damage show itself in the previously formed eggs or only in the upper part of the ovarioles, and what form does it take?

C. TOROSSIAN: I have not yet studied the ovaries of irradiated females, having preferred to follow the development of these queens throughout the whole of their life-span. Their behaviour and biology was our first objective, though we do eventually intend to make a histo-cytological study of irradiated queens.

REARING TECHNIQUE, BIOLOGY AND STERILIZATION OF THE COFFEE LEAF MINER, Leucoptera coffeella Guer. (LEPIDOPTERA: LYONETIIDAE)

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Abstract

REARING TECHNIQUE, BIOLOGY AND STERILIZATION OF THE COFFEE LEAF MINER, Leucoptera Coffeella Guer. (LEPIDOPTERA: LYONETIIDAE). For two years the authors studied the feasibility of controlling the coffee leaf miner Leucoptera coffeella Guer. by the radiation sterilization technique. During this period a technique for raising large numbers of this insect on potted coffee plants in the laboratory was devised. The optimal range for the development of egg, larval and pupal stages of the coffee leaf miner was between 20 and 30°C. The pupal stage of female insects was slightly shorter than that of the male. The laying of fertile eggs began during the first night following emergence. During an oviposition period of 16 days the average fecundity was 68 eggs. The maximum oviposition by a single female was 131 eggs over the life span while as many as 34 eggs were laid during a single day of oviposition. To investigate the best stage to induce radiation sterilization, pupal and adult insects were irradiated with ^{60}Co gamma rays. Seven-day pupae (close to emergence) showed 88% lethality in males when given 60 krad; the survivors retained some fertility. Adult females receiving 70 krad were 100% sterile while males given 90 krad showed 0.02% fertility. Doses as high as 90 krad given to newly emerged adults did not reduce longevity. Studies are continuing to determine if sterilizing doses impair sexual vigour and mating competitiveness of the treated males.

1. INTRODUCTION

The coffee leaf miner, Leucoptera coffeella Guer., is one of the great destructive pests of coffee plantations in most coffee-growing countries of the Western hemisphere. Recently it has become a very serious pest of coffee in Central American countries, especially in Guatemala [1-3] and Costa Rica [4]. The damage is done by the larvae which feed on the mesophyll layer of the leaves. The heavily mined leaves are easily dislodged, and under severe infestation the plants may be completely defoliated.

The successful eradication of the screw-worm fly, Cochliomyia hominivorax (Coquerel), from Curacao [5] and southeastern portions of the United States [6] by mass release of gamma sterile males has demonstrated the great potential of this new method of insect control. Currently research is underway on several insect pests in various laboratories of the world to study the feasibility of the sterile-male technique for control and eradication of these pests. The exploratory studies to evaluate the gamma sterile-male technique for controlling the coffee leaf miner started in our laboratory 2 years ago. The preliminary results of these studies are presented in this paper.

2. REARING TECHNIQUE

A continuous laboratory supply of adequate numbers of leaf miners in all stages is essential for carrying out sterilization studies. Techniques to rear the coffee leaf miner continuously in large numbers in a laboratory have not been worked out previously. Our first attempt in this study, therefore, was to find a satisfactory way to rear this insect in the laboratory. We have developed a method which allows continuous mass rearing of this insect on living coffee plants.

A number of pupae were collected from the infested coffee field. Upon adult emergence, the moths were transferred to an oviposition cage 24" x 22" x 18" containing living coffee plants (Fig. 1). The four sides and top of the cage were covered with 52-mesh plastic screen. The bottom was made of plywood divided into four rectangular sections. Each section had a 9" x 9" window which could be opened or closed by a sliding plywood panel. These panels were opened to introduce the coffee plants into the cage. On the inner edge of each panel there was a 3/4" semicircular hole that coincided with a similar hole in the bottom frame of the cage when the bottom panel was closed.

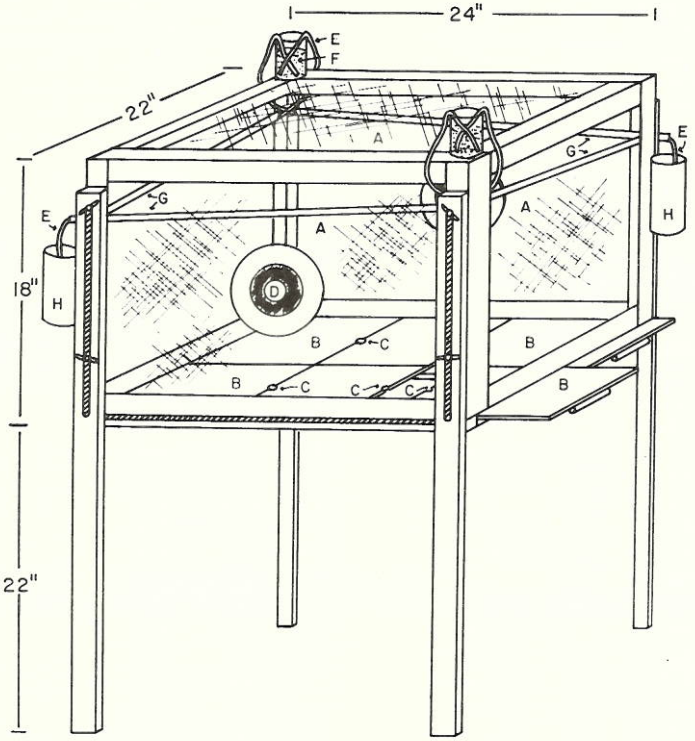

FIG. 1. Oviposition cage used for rearing the coffee leaf miner, Leucoptera coffeella Guer.

A = 52-mesh plastic screen
B = Sliding plywood panel
C = Plant stem hole
D = Opening for introduction of adults
E = Paper cellulose wick
F = Sugar solution
G = U-shaped stainless steel channel
H = Vessel for collecting excess sugar solution

The stem of the coffee plant used for oviposition passed through this hole. The cage stood on four legs which could be adjusted to a maximum of 30" depending upon the height of the coffee plant. The design of the cage permitted the manipulation of the coffee plants so that only the upper foliar portion of the coffee plant was available for oviposition. The soil pot and the lower part of the coffee plant were outside the cage. This arrangement allowed more space for moths and coffee leaves. A system was devised to provide the moths with plenty of fresh sugar solution. Around the inside of the cage, four paper cellulose wicks were supported on four U-shaped stainless steel channels placed near the top. The wicks had been soaked in a sugar solution with one end inserted into a beaker of sugar water placed on top of the cage. The other end of the wick was placed over an empty vessel to collect any excess solution. Fresh sugar solution was added daily, and the wicks were changed weekly to insure the moths ample food. The adult moths emerging from the pupal holding cages were transferred into the oviposition cage through a circular hole on either side of the cage.

The pupal holding cages were made of wooden frames covered with heavy black cloth. Emerging moths attracted to light were collected daily in glass beakers inserted at one end of the cage. The cages were stored in a battery of steel frame racks.

Four young coffee plants (10-12 months old) were placed in each oviposition cage containing 2000-3000 moths. Oviposition was allowed for 2 days before the plants were replaced. It is recommended that the plants be changed every day to obtain pupae of uniform age and to increase insect production. By leaving the plants for 2 days in oviposition cages, sometimes the plants were killed by heavy larval infestation resulting from excessive oviposition. In such cases the larvae died before pupation.

The coffee plants infested with Leucoptera eggs were placed in the laboratory at atmospheric conditions of 20-27° C and 80-90% relative humidity for 17 days. During this period the larvae matured in the leaves and began pupation. Within 4 or 5 days most of the full-grown larvae had left the mines, dropped to the lower leaves by a silken thread, and pupated in a silken cocoon. Coffee leaves with pupae were removed from the plants and incubated in pupal holding cages. Moths began to emerge on the 2nd day with the majority of them emerging during the following 4 to 5 days. The adults were transferred to oviposition cages daily.

Approximately 2,000 moths were produced daily with this rearing technique using three oviposition cages and 12 coffee plants every 2 days of oviposition. It would be possible to increase production to any desired level by augmenting the rearing facilities.

3. BIOLOGICAL STUDIES

Information on the biology and ecology of an insect is essential for evaluation of its control by the sterile-male technique. Studies have been carried out in our laboratory on some biological phases of this insect. All the insects used in the experiments were

reared on potted coffee plants in the laboratory. Temperature-controlled experiments were carried out inside the constant-temperature cabinets. Humidity was not controlled except in the case of pupal studies.

The effects of various temperatures on the rate of development and hatchability of the coffee leaf miner eggs are presented in Table I. The results indicated that the optimum temperature for incubation of the eggs was 20-30° C. Eggs incubated at 30° C gave highest percentage of egg hatch (95.0%) and the shortest (average of 4.0 days) incubation period. At 35° C larval hatch from the eggs was normal but only 8.5% of the larvae were able to mine the leaves. The rest of the newly emerged larvae died on the outer surface of the leaf. The reason for this high larval mortality is not known. Excessive heat (35° C) probably kills the newly emerged larvae before they are able to penetrate the epidermis of the leaf.

Table I

Temperature effect on the rate of development and hatchability of the coffee leaf miner, Leucoptera coffeella Guer., eggs

Temperature °C	Number of eggs observed	Percent hatch	Development periods (days)		
			Max.	Min.	Avg.
15*	339	0	0	0	0.0
20	855	80.4	13	10	10.7
25	588	81.1	7	5	5.5
30	806	95.0	5	4	4.0
35	282	8.5	5	4	4.1

*Incubated for 60 days.

Data in Table II show that the optimum temperature range for the development of the coffee leaf miner larvae lies between 25-35° C. Larvae reared at 30° C yield the highest percentage of pupae (92.7%). At 35° C the larval development was fastest. The average larval period at 35° C was 7.2 days. Larvae reared at 15° C fail to pupate although 2.7% of them matured and left the mines. The larvae of all the treatments were reared on isolated coffee leaves except in treatments of 15 and 35° C whose larvae were reared on potted coffee plants.

Table III shows the effect of various temperatures on the rate of development of the coffee leaf miner pupae. Temperature between 20° and 30° C was optimum for incubation of the pupae. The adult emergence from the pupae incubated at this range of temperature

Table II

Effect of temperature on the development of the coffee leaf miner, Leucoptera coffeella Guer., larvae

Temperature °C	Number larvae reared	Percent pupation	Larval period (in days)		
			Max.	Min.	Avg.
15	564	2.7*	87	68	73.6
20	111	82.2	29	20	23.1
25	92	91.9	16	12	13.1
30	254	92.7	11	8	9.0
35	295	88.5	8	7	7.2

*Larvae left the mines but failed to pupate.

Table III

Effect of temperature on the development of the coffee leaf miner, Leucoptera coffeella Guer., pupae*

Temperature °C	Number pupae observed	% moth emergence	Pupal period in days		
			Max.	Min.	Avg.
15	150	34.7	38	30	33.3
20	100	95.0	16	13	13.7
25	100	97.0	7	5	5.3
30	251	88.8	7	5	5.3
35	100	33.0	6	5	5.5

*Incubated at 100% RH.

varied from 88.8 to 97.0%. Incubation of the pupae at 25° C gave highest percentage moth emergence (97.0%) and shortest pupal period (5.3 days).

Results summarized in Table IV indicate that in the coffee leaf miner, the female pupal period was a little shorter than the male pupal period. The average pupal period at 25° C temperature and 50 to 60% RH for the female and male was 6.7 and 7.1 days, respectively. Female moths emerged 1 day earlier than male moths.

Table IV

Duration of male and female pupal periods* of the coffee leaf miner, Leucoptera coffeella Guer.

Pupal period (days)	Percent adult emergence	
	Male	Female
6	0	34
7	92	66
8	8	0

Average pupal period: Male = 7.1 days and Female = 6.7 days.
Total number of pupae observed = 860.

*At 25° C and 50-60% relative humidity.

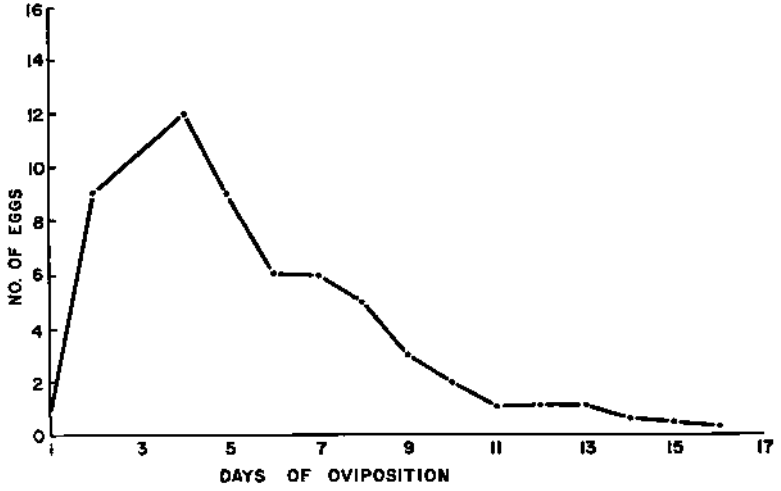

FIG. 2. Oviposition rate of the coffee leaf miner, Leucoptera coffeella Guer.

Oviposition rate of female coffee leaf miner was studied in small cages by single-paired matings. Daily egg collection was made from each female for a period of 16 days. At the end of this period most of the moths had either died or stopped egg production. The female moths started laying fertile eggs on the first night of emergence. The rate of oviposition increased rapidly reaching a maximum on the 4th day. After the 4th day the oviposition declined sharply until the 7th day and then gradually diminished to almost

zero by the 16th day (Fig. 2). Thus the female coffee leaf miner moth lays a majority of her eggs during the 1st week of adult life. A female lays an average of 68 eggs during her entire life. The maximum oviposition per female was 131 eggs with 34 eggs the maximum number laid by a female on any 1 day.

4. STERILIZATION STUDIES

Pupae of uniform age for irradiation studies were obtained by spreading small coffee twigs with leaves below the infested coffee plants in the morning between 7:00 and 8:00 A.M. Full-grown larvae left the mines and pupated on these leaves. The twigs were removed 8 hours later in the evening. Thus the age of the pupae used in the irradiation tests varied from 0-8 hours.

The sterilization studies were started by irradiating late-stage pupae (7 days old). The presence of mature sperm was noticed in such pupae. A series of initial irradiation tests conducted on late-stage pupae indicated that the pupal stage of the coffee leaf miner was not suitable for inducing sterility in the adult moths. Radiation dose of 60 KR applied to 7-day-old pupae (close to adult emergence) was lethal (Table V). The adverse effect of irradiation was more pronounced on male pupae than female pupae. Pupae irradiated with 60 KR produced 29.3% male moths compared with 45.5% male emergence from untreated pupae. The female emergence was normal (41.2%) from pupae irradiated with 60 KR. The majority of the male moths that emerged from irradiated pupae were sluggish and unable to fly normally. Within 48 hours after the adult emergence 88.1% males were dead. Fertility experiments carried out with the surviving moths indicated that they were not 100% sterile. In subsequent tests, therefore, radiation was applied during the adult stage. The reason for the severe lethal effect of radiation on male pupae and no or little effect on female pupae is probably that at the time of treatment female pupae are further developed than male pupae.

Newly-emerged moths were irradiated with several doses of gamma radiation at a dose rate of approximately 2414 R/min. The treated insects were confined in cages together with virgin moths of the opposite sex. Each cage had 20-25 pairs of moths. Since sufficient numbers of insects were not available at one time, the experiment was completed in several steps. A control was used for each experiment. Daily egg collection was made for a period of 7 days. The results of these experiments are summarized in Table VI. The data indicated that the sterilization dose of this insect was 90 KR. Male moths were more radioresistant than female moths (when sterility is taken as the index of the radiosensitivity). A radiation dose of 70 KR induced 100% sterility in the treated females whereas the males receiving 90 KR still retain 0.02% fertility. The high radioresistance in Lepidoptera is not uncommon. The sterilizing dose for the codling moth, Carpocapsa pomonella L., is reported to be 40,000 rads [7]; the tobacco budworm, Heliothis virescens - 35,000-45,000 rads [8]; and for the Indian meal moth, Plodia interpunctella (Hübner), and the Angoumois grain moth, Sitotroga cerealella (Oliver), as high as 100,000 rads [9].

Table V

Effect of gamma radiation (60 KR) applied to 7-day-old pupae on the adult emergence and mortality of the coffee leaf miner, Leucoptera coffeella Guer.

Treatment	Number of pupae studied	Percent moth emergence		Percent moth mortality*	
		Male	Female	Male	Female
Normal	1,030	45.5	39.1	0.0	0.0
Irradiated	1,030	29.3	41.2	88.1	23.6

*Within 48 hours after adult emergence had started.

Table VI

Effect of gamma radiation* on the fertility of the coffee leaf miner, Leucoptera coffeella Guer.

Dosage (KR)	Matings** Female X Male	No. pairs	Number of eggs Examined	Number of eggs Hatched	Percent fertility
Check	N X N	254	13,932	11,169	80.17
60	N X R	70	4,022	268	6.66
	R X N	70	1,861	2	0.11
	R X R	70	2,096	0	0.00
70	N X R	70	3,434	109	3.17
	R X N	70	944	0	0.00
	R X R	60	1,248	0	0.00
80	N X R	70	4,216	57	1.35
	R X N	70	667	0	0.00
	R X R	65	1,495	0	0.00
90	N X R	75	4,510	1	0.02
	R X N	75	560	0	0.00
	R X R	75	1,279	0	0.00

*Irradiated at adult stage. Age of the moths at the time of treatment varied a few hours to 24 hours in all the crosses except those having both sexes irradiated. Females of the crosses (R female X R male) were approximately 48 hours old and were laying normal viable eggs at the time of treatment.

**The letters R and N designate irradiated and normal moths, respectively.

Since the induction of sterility in this insect requires the application of radiation at the adult stage, it is quite likely that some mating occurred before the radiation was applied. Therefore, a mixed population of normal moths that had laid fertile eggs was irradiated and the subsequent fertility studied. The crosses (R female X R male) in Table VI constitute such treatments. The results indicated that a dose of 60 KR induced 100% sterility in females that had laid normal fertile eggs before irradiation.

It seems that the dose of 90 KR (applied to newly-emerged moths) does not adversely affect the longevity of the treated moths. We are planning to study the sexual vigor and the mating competitiveness of the sterile males in the near future.

REFERENCES

[1] FISCHER, K.U., HERRERA, J.C., Minador del café, estudio técnico en Guatemala, Villanueva, Escuela Nacional de Agricultura (1963) 5.
[2] HERNANDEZ, M.P., Revta Cafet. (Guatemala) 31 (1964) 7-17.
[3] HAMILTON, D.W., J. econ. Ent. 60 5 (1967) 1409-13.
[4] HAMILTON, D.W., unpublished report, USDA, ARS, Entomology Research Division, Vincennes, Indiana (1964).
[5] BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, D.E., NEW, W.C., DUDLEY, F.H., BUSHLAND, R.C., J. econ. Ent. 48 4 (1955) 462-68.
[6] LINDQUIST, A.W., "Use of sexually sterile males for eradication of screw-worms," in Radioisotopes and Radiation in the Life Sciences, 2nd Inter-American Symposium on the Peaceful Application of Nuclear Energy, Buenos Aires 1959, TID-7554, Washington, D.C., Pan-American Union (1960) 229-35.
[7] PROVERBS, M.D., NEWTON, J.R., Can. Ent. 94 11 (1962) 1162-70.
[8] FLINT, H.M., KRESSIN, E.L., J. econ. Ent., in the press.
[9] COGBURN, R.R., TILTON, E.W., BURKHOLDER, W.E., J. econ. Ent. 59 3 (1966) 682-85.

DISCUSSION

J.E. SIMON: I congratulate you and your colleague on the technique you have devised. One point I am not clear about is: according to Tables I, II and III the maturation period of the moth L. coffeella at 30° C is 4.0 + 9.0 + 5.3 = 18.3 days, so how, with only three cages, can you maintain continuous production of adult moths?

K.P. KATIYAR: At first, of course, production is not continuous, but as soon as the moths hatched from eggs laid on the first day of oviposition reach maturity the cyclic process is sustained. Since there is oviposition daily on the coffee plants there is moth emergence daily.

E.M. SHUMAKOV: How much does it cost to rear, say, one million moths in the laboratory?

K.P. KATIYAR: I don't believe we ever tried to work out the exact cost of rearing one million insects, but I imagine that expenditure on coffee plants would come to somewhere around $40-$60 per million moths. In this work we really were not trying to find a cheap rearing method but rather to breed the insect successfully in the laboratory for our own experimental purposes.

M. COHEN: Are there any data on the levels of population of L. coffeella in the field, and if these are very high could your rearing process give a throughput which would enable you to swamp the numbers of wild moths?

K. P. KATIYAR: The field population of L. coffeella is not known. Our present rearing method is certainly not sufficient for making large-scale sterile insect releases; our aim in this work was rather to develop some technique which would provide us with enough insects to do sterilization studies in the laboratory.

M. COHEN: Does L. coffeella mate more than once?

K. P. KATIYAR: I have no definite information on this but I believe that it does.

C. F. CURTIS: I think the question of multiple matings of the females may actually be rather important because of the very high dose – 90 krad – that you required to induce complete sterility. I believe I am right in saying that in Habrobracon, where dominant lethal mutations can be readily distinguished from sperm damage impairing fertilizing capacity, 90 krad induces a considerable amount of such damage. If the same applies to your moth, L. coffeella, it might happen that a female which had mated with several sterile males and one fertile male would show full fertility.

K. P. KATIYAR: All I can add is that we have not yet studied the damaging effect of 90-krad doses on sperm. All we have done so far is to determine that dose which completely sterilizes the males and this is found to be 90 krad. I realize that it is quite a high dose and there is every possibility that it would cause some sperm damage. We are definitely planning to investigate the effect of 90 krad on the sexual vigour and mating ability and hence on the competitiveness of sterile males with normal males, and if this work indicates that damage, such as sperm inactivation, is caused, then certainly the multiple mating habits of the female will be a very important factor, as you have pointed out. In such a case one normal mating would cancel out all the previous sterile matings since the sterile sperm will not contribute to fertilization of the egg. On the other hand, if future studies indicated that the sterilizing dose of 90 krad did not induce sperm damage or, in other words, that the sterile sperm is fully competitive with normal sperm, then the multiple mating habits of the female would not hamper attempts to control this insect by the sterile-male technique – provided, of course, that the other factors were favourable.

D. T. NORTH: I should like to comment briefly on two points. Firstly, I do not believe that in practice as much as 90 krad is required to accomplish sterilization of the coffee leaf miner, since fertility is well under 10% at 60 krad. For the purposes of a sterile-male release programme, this is a sufficiently high degree of sterility; furthermore, the sizable drop in required dosage will most probably result in reduced physiological and somatic damage to the insects.

Secondly, concerning Dr. Curtis's comment on the effect of multiple mating, from our own work with Trichoplusia ni, we have found that the irradiated male tends to transfer less sperm or at high doses none at all. In cases like this, multiple mating becomes an important factor since normal males in the population are continuing to contribute normal quantities of sperm. In practice this means that normal matings following

sterile-male matings would cancel out the effect of releasing the irradiated males.

K. P. KATIYAR: I am not convinced that 90% sterility is an acceptable practical level for the coffee leaf miner in a sterile-male release programme. If it is, then a dose of 60 krad would certainly be much more acceptable in view of the reduction in somatic damage caused to the insect.

As to your second point, I agree with what you say but cannot elaborate on this since we have not in fact carried out any studies on the coffee leaf miner to test the effect of sterilizing dose on the sexual vigour or mating competitiveness of the treated males.

V. LABEYRIE: Is the behaviour of irradiated males different from that of unirradiated ones? What selection process is followed by normal females when irradiated and normal males are in competition with each other?

K. P. KATIYAR: I cannot yet say what kind of selection a normal female would make in the presence of both normal and irradiated males but, looking at cases of other Lepidoptera in the literature, I should imagine that the sterile males would be less competitive.

V. LABEYRIE: In the case of mating with an irradiated male, are spermatozoa transferred to the spermathecae or, if not, are some secretions from the paragonia suppressed so that the males are rendered sterile although they can still produce spermatozoa?

K. P. KATIYAR: We have not checked the spermathecae for the presence of sperm in a female mated with irradiated males. It would certainly be interesting to study the effect of sterilization on the secretory activity of the male reproductive system and it is just possible that the treated male does possess motile sperm but that this is not transferred to the female during mating because the treated male lacks seminal fluid, which acts as a carrier in the transfer of spermatozoa from male to female. It is quite likely that production of this seminal fluid is affected by the sterilizing radiation dose.

B. NAGY: Did you make any observations on the effect of predators or parasites during your mass rearing process? I should have thought that since you used living coffee trees you might well have found some predators or parasites in the rearing cages.

K. P. KATIYAR: We did not observe any parasites or predators affecting our moth production other than ants, which present a threat to the insect in the egg and pre-pupal stages. We smeared the legs of the rearing cages with tangle foot in order to repel the ants.

C

ISOTOPE APPLICATIONS: PHYSIOLOGY AND BIOCHEMISTRY

(Session V, Part 1)

Chairman: L.K. CUTKOMP
UNITED STATES OF AMERICA

UPTAKE OF ISOTOPICALLY LABELLED INSECTICIDES IN RELATION TO TEMPERATURE AND OTHER PHYSICAL FACTORS *

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Abstract

UPTAKE OF ISOTOPICALLY LABELLED INSECTICIDES IN RELATION TO TEMPERATURE AND OTHER PHYSICAL FACTORS. Labelling insecticides with isotopes provides a sensitive method for investigating their uptake in relation to unusual toxic responses in insects. The uptake of ^{14}C-DDT was traced at three different exposure temperatures with mosquito larvae, Aedes aegypti. In addition, explanations of different toxicity responses related to the insect densities were studied with ^{14}C-DDT on Tribolium confusum, and with tritium-labelled nicotine on mosquito larvae. In the latter study, the labelled nicotine produced greater toxicity which was apparently due to a more stable chemical bonding resulting in failure of the insects to metabolize the isotopically labelled chemical as readily as natural nicotine.

1. INTRODUCTION

The uptake or 'pick-up' of an insecticide is one of the critical factors leading to the ultimate toxic effect on an insect. The metabolism of a compound, either degradation or activation, is the second major component influencing the toxic response. The present review is limited to the uptake of isotopically labelled insecticides, particularly as related to temperature differences and population density during the exposure period.

The extremely sensitive detectability level aids in explaining unusual or unexpected responses to a toxin. Examples are presented as related to temperature and to the physical factor of population density. These factors become highly important when, with certain insecticides, the insect response does not follow the predicted pattern.

2. EXPOSURE TEMPERATURE

DDT exhibits a negative temperature coefficient [1-4]. One plausible explanation is that the uptake or sorption of the toxin is greater in the cuticle of an insect at a cooler temperature [5]. Since the stability of DDT was well known in addition to that of the possible metabolites, DDE and possibly TDE, a ^{14}C labelling of the trichloroethane portion of the molecule was used to determine uptake at three discrete temperatures, 10, 20 and 30°C [6]. The results showed that the uptake of ^{14}C-DDT by

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Aedes aegypti larvae was positively correlated with temperature, and thus did not correspond to the negatively correlated toxicity responses. The uptake of ^{14}C-DDT was 41.5, 118.3, and 157.4 μg/mg dry weight at exposure temperatures of 10, 20 and 30°C, respectively. Of additional interest were the comparative uptakes by the heads, thoraces, and abdomens of larvae as tabulated in Table I. The heads had the greatest uptake of ^{14}C-DDT at the cooler temperature, while the thoraces had the greatest uptake at the warmer temperatures. Thus the phenomenon of the negative temperature effect, particularly evident with DDT and related insecticides, but not with many other insecticides, does not appear to be attributable to the uptake of DDT.

TABLE I. RADIOASSAY OF COMPONENTS OF ^{14}C-DDT-TREATED Aedes aegypti LARVAE

The quantity (μg × 10^{-4} per mg dry wt.) of ^{14}C-DDT determined from heads, thoraces, and abdomens. Larvae exposed to 0.003 ppm DDT for 48 hours. Values are means of three replications involving 50 larvae each

Larval component	Treatment temperatures (deg C)		
	10°	20°	30°
Head	23.56	40.93	56.40
Thorax	12.70	57.06	77.33
Abdomen	5.23	20.26	23.53
Total larvae	41.49	118.25	157.36

3. POPULATION DENSITY

Isotopically labelled insecticides were used for elucidation of differential responses to a toxin as related to population density. Deviations apparently due to numbers were apparent with assays of mosquito larvae, Aedes aegypti and Anopheles quadrimaculatus, and with flour beetles, Tribolium confusum. Early research on DDT toxicity to mosquito larvae [7, 8] revealed that changing the number of larvae per unit volume by varying either the volume or the number might affect the per cent mortality. Results indicated that the greater the number of larvae the lower the per cent mortality. This was later substantiated by Schmidt and Weidhaas [9] who utilized isotopically labelled insecticides to solve a problem. They found a greater absorption (or uptake) of ^{14}C-DDT and a higher mortality with lower densities of Anopheles quadrimaculatus larvae than with higher densities. Comparisons with two ^{32}P-labelled organophosphates, coumaphos (Bayer 21/199) and dimethoate (A.C. 12880) did not show this phenomenon to be related to the density of mosquito larvae. In brief, both per cent mortality and uptake

or absorption of the dose were approximately the same even when the number of larvae per beaker was changed from 25 to 400 (Table II). Furthermore, by calculating amounts of labelled insecticides removed from the treated water it was possible to conclude that the variation in mortality depended upon the per cent removal of the insecticide, which was critical in the case of DDT, but not critical with the two organophosphates, since the concentration of the active toxins was not decreased enough to affect the mortality response.

One cannot conclude, however, that population density may not be a factor in the toxicity responses of organophosphates. In a study with parathion, e.g. the LD_{50} value with 10 larvae per beaker was about half (6.4 ppb)[1] that when 300 larvae were tested (LD_{50} = 11.3 ppb) [10] (Table III).

The uptake of ^{14}C-DDT was also used to help clarify results of a bioassay of different densities of the confused flour beetle, Tribolium confusum [10]. These results contrasted with those of the mosquito larvae bioassay in that a positive correlation was found between density and per cent mortality. The relationship was only evident in covered petri dishes. The experiment also differed in that the amount of DDT used for surface exposure of the beetles was always in excess, while in mosquito larval tests DDT was depleted by a high density of larvae. The use of ^{14}C-DDT did not reveal significant differences in uptake of the labelled insecticide related to density, and the conclusions drawn were that a critical shortage of oxygen occurred with high densities of beetles tested in covered dishes, thus resulting in greater mortality when they were exposed to DDT.

Tritium-labelled nicotine produced rather unexpected results when tested on Aedes aegypti larvae [11], and these should serve to warn

TABLE II. TWENTY-FOUR HOUR MORTALITY, ABSORBED DOSE, AND PER CENT OF TOTAL AVAILABLE INSECTICIDE REMOVED BY FOURTH-INSTAR Anopheles quadrimaculatus LARVAE EXPOSED IN 250 ml OF WATER TREATED WITH RADIOACTIVE DDT (0.007 ppm) AND COUMAPHOS (0.03 ppm)

Number of larvae	Per cent mortality		Absorbed dose (µg/Larva)		Per cent removed	
	DDT	coumaphos	DDT	coumaphos	DDT	coumaphos
25	55	53	0.0069	0.0024	9.9	0.97
50	33	48	0.0064	0.0023	18	1.5
100	14	54	0.0046	0.0020	26	2.7
200	3	53	0.0033	0.0018	38	4.8
400	3	53	0.0026	0.0020	59	10

[1] ppb = parts per billion (10^9).

TABLE III. THE LD_{50} VALUES DETERMINED FOR PARATHION AND 4th INSTAR LARVAE OF Aedes aegypti WITH DIFFERENT NUMBERS OF LARVAE FOR EACH TEST

Numbers of larvae in 100 ml per beaker	LD_{50} in ppb
10	6.4 ± 0.3
25	7.4 ± 0.2
100	7.6 ± 0.2
200	8.6 ± 0.1
300	11.3 ± 0.2

researchers against blindly assuming that the behaviour of an isotopically labelled compound would be the same as that of natural chemicals. As with DDT, preliminary studies showed differences in larvae mortality related to density. The relationship was a negative one, as was previously reported for DDT and parathion, although the magnitude of the difference was less. The likely explanation was depletion of nicotine due to greater numbers of larvae. Tritium-labelled nicotine was used to test this hypothesis. The radioactive compound was prepared from nicotinic acid 2-^{3}H which was introduced into the nutrient solution of the roots of Nicotiana tabacum plants to obtain radioactive nicotine (nicotine 2-^{3}H) biosynthetically. The use of the tritium-labelled nicotine resulted in no differences in response related to different densities of the mosquito larvae. In addition to this finding, radioactive nicotine was significantly more toxic to the larvae than natural nicotine (Fig. 1). The LD_{50} values for tritiated nicotine were 178 and 175 ppm with larval populations of 50 and 400, respectively. The LD_{50} values for natural nicotine were 202 and 275 ppm with larval populations of 25 and 400, respectively. Several possible explanations of the increased toxicity were ruled out, including possible contamination of a by-product and the liberation of radioactive agent. The latter possibility was tested by using tritiated water at a much higher level of radioactivity (1 mCi/100 ml water) with no mortality following a 24-hour exposure period. The best explanation of increased toxicity appears to be the very slow metabolic decomposition of tritiated nicotine, the cleavage of the carbon-tritium bond being much more difficult than the non-isotopic carbon-hydrogen bond.

The difference must be related to the site of labelling (the 2-position on the pyridine ring of the nicotine molecule), thus involving a portion of the nicotine molecule (pyridine ring) which had not been considered in previous studies of nicotine metabolism, either with insects or vertebrates.

The experimental study with nicotine emphasizes the value of isotopically labelled compounds, but sounds a word of caution regarding assumptions that the activity of naturally occurring compounds is the same as that of their isotopically labelled counterparts.

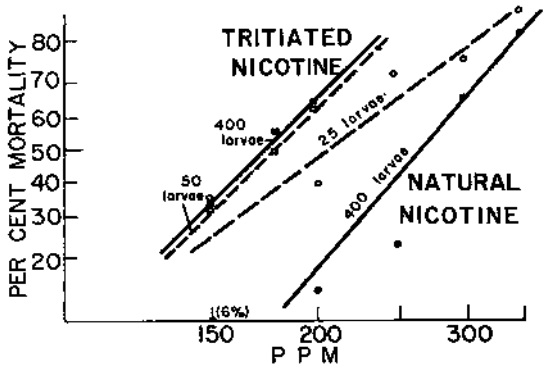

FIG. 1. Regression lines showing 48-hour responses of larvae of Aedes aegypti to natural nicotine and tritiated nicotine. (adapted from Makky, Doctoral thesis, Univ. of Minn., 1965)

4. SUMMARY

Isotopically labelled insecticides can be detected at very low levels, and are thus very useful for determining uptake of the insecticide by the insect from the environment. One laboratory example is the uptake of ^{14}C-DDT by mosquito larvae at three environmental temperatures, 10°, 20° and 30°C. A positive correlation occurred between uptake and increase in temperature. However, the toxicity response was negatively correlated with temperature. ^{14}C-DDT was also used to analyse differential toxicity responses when the population density was varied. A lower per cent mortality occurred with higher densities of mosquito larvae. The effect was due to a greater depletion of the toxin by greater total numbers of larvae, resulting in less toxin per larva. A contrasting study with flour beetles revealed a higher per cent mortality with higher densities in covered petri dishes. Since the uptake of ^{14}C-DDT did not differ at different densities, a distinct cause was indicated. The effect appeared to be due to oxygen depletion associated with DDT toxicity.

One additional study revealed that natural nicotine was depleted from solution at a higher rate by greater densities of mosquito larvae, an effect corresponding to that described for DDT. However, when tritiated nicotine was employed, an increased toxicity occurred unrelated to population density. Evidence indicates the inability of larvae to metabolize the isotopically labelled compound significantly during the exposure period.

REFERENCES

[1] FAN, H.Y., CHENG, T.H., RICHARDS, A.G., The temperature coefficient of DDT action in insects, Physiol. Zool. 21 (1948) 48.

[2] VINSON, E.B., KEARNS, C.W., Temperature and the action of DDT on the American roach, J. econ. Ent. 45 (1952) 484.

[3] LOFGREN, C.S., CUTKOMP, L.K., Toxicity of DDT to the American cockroach when lipid content and temperature are varied, J. econ. Ent. 49 (1956) 167.

[4] DAS, M., NEEDHAM, P.H., Effect of time and temperature on toxicity of insecticides to insects. I: Tests of DDT on larvae of Aedes aegypti, Ann. appl. Biol. 49 (1961) 32.

[5] RICHARDS, A.G., CUTKOMP, L.K., Correlation between the possession of a chitinous cuticle and sensitivity to DDT, Biol. Bull. 90 (1946) 97.
[6] ZUBAIRI, M.Y., CUTKOMP, L.K., The pick-up of C^{14}-DDT at different temperatures. Entomologia exp. appl. 7 (1964) 239.
[7] KRUSE, C.W., LUDVIK, G.F., HAWKINS, W.B., Factors affecting evaluation of insecticides against Anopheles larvae, J. econ. Ent. 45 (1952) 598.
[8] WATTAL, B.L., The influence of different numbers of insects on mortality produced by insecticides, as shown with Tribolium spp. and Aedes aegypti L., Diss. Abstr. Publ. No. 3965, Univ. of Minn. (1952).
[9] SCHMIDT, C.H., WEIDHAAS, D.E., Absorption and toxicity of three radioactive insecticides in larvae of two species of mosquitoes, J. econ. Ent. 51 (1958) 640.
[10] KOLKAILA, A.M., CUTKOMP, L.K., unpublished data, Department of Entomology, Fisheries, and Wildlife, Univ. of Minn. (1963).
[11] WATTAL, B.L., CUTKOMP, L.K., The relationship between density and mortality of flour beetles exposed to insecticide-treated surfaces, J. econ. Ent. 52 (1959) 1184.
[12] MAKKY, A.M., Radioactive nicotine: biosynthesis, toxicity, and metabolism, Diss. Abstr. No. 66-9030 (1965).

DISCUSSION

F.T. PHILLIPS: What do the quantities shown in Table I represent in terms of toxicity? For instance, is there any correlation between (a) the uptake at each of the three temperatures, either considering the various body components separately or the larva as a whole, and (b) a chosen toxicity level (LD_{50}, LD_{90}, etc.) at the particular temperature which is being considered?

L.K. CUTKOMP: The values in Table I were determined after treatment at a lethal concentration somewhat less than LD_{50} or LC_{50}. This concentration was chosen deliberately, since below the LC_{50} level a stronger expression of negative response is exhibited and at higher levels such as LC_{90} the amount of DDT involved would probably be excessive.

F.T. PHILLIPS: Did you measure rates of uptake over the 48-hour period and, if so, were these the same throughout the period?

L.K. CUTKOMP: We did obtain some measurements after 24 hours and, as I recall, the relationships at the various temperatures were about the same.

F.T. PHILLIPS: With reference to Table II, does the lower solubility of DDT in water compared with that of coumaphos in water have any bearing on the observed greater percentage removal of DDT, considering that DDT in aqueous solution accumulates at interfaces, such as air/water, container walls/water, and insect cuticle/water?

L.K. CUTKOMP: Both the low solubility of DDT in water and its great affinity for lipids or lipoproteins in the insect cuticle seem to be involved. Coumaphos is far less lipophilic and it would thus appear that the mosquito larvae can remove DDT from the aqueous suspension much more readily than an organophosphate.

I.A. KANSU: I am curious about the amount of DDT taken up as given in Table I. Do these data refer to the amount found in the body of the mosquito larvae or that found both in and on the body? Secondly, am I right in thinking that Table II in fact shows 'observed mortality' rather than 'corrected mortality' based on a process such as the Abbot formula?

L.K. CUTKOMP: The amount reported in Table I is that quantity found within the insect, since the outside of the larvae was rinsed with acetone before analysis was made. As to your second question, I would suggest you refer to the paper by Schmidt and Weidhaas (Ref.[9] of my paper).

P.A. LANGLEY: It was stated that cleavage of the carbon-tritium bond in tritiated nicotine is more difficult than cleavage of the non-isotopic carbon-hydrogen bond. Have you thought of using ^{14}C-labelled tritiated and non-tritiated nicotine to substantiate this statement quantitatively?

L.K. CUTKOMP: Yes, we considered using ^{14}C, but it would not be possible, I think, to prepare the nicotine ^{14}C biosynthetically as was done for the tritiated nicotine. We have not yet gone into this problem in any detail, but I am of the opinion that substitution of deuterium instead of tritium would make for a better comparison.

P. PELEGRIN: It would be interesting to determine whether the toxicity of deuterated nicotine lay between that of unlabelled and tritiated nicotine, the mass of ^{2}H being intermediate between those of ^{1}H and ^{3}H.

L.K. CUTKOMP: Certainly, substitution of deuterium for ^{1}H or ^{3}H would be most appropriate for purposes of comparison in cases where chemical bonding at the same site could be determined and the biological activity compared.

H. MARCHART: As regards an explanation for the negative temperature coefficient you found for response to DDT, have you envisaged the possibility of a change in the rate of dehydrochlorination by the insect?

L.K. CUTKOMP: Yes, this had been considered, but all chemical tests show that dehydrochlorination proceeds more rapidly with increasing temperature. It seems more likely, therefore, that a chemical explanation would involve certain metabolic compounds and their varying proportions within the insect.

METABOLISM OF MALATHION IN THE DESERT LOCUST Schistocerca gregaria FORSKAL: Distribution and degradation of ^{32}P-labelled malathion in vivo

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Abstract

METABOLISM OF MALATHION IN THE DESERT LOCUST Schistocera gregaria FORSKAL: DISTRIBUTION AND DEGRADATION OF ^{32}P-LABELLED MALATHION IN VIVO. Distribution and degradation of malathion in the desert locust (Schistocera gregaria) was studied in vivo with ^{32}P-labelled malathion. Results showed that 65.6% of the applied dose was absorbed by the locust treated with 100 µg malathion/insect, within 270 minutes. During the same period, the locust excreted 24.5% of the total applied dose, or nearly 37% of the absorbed malathion, in faeces. The major amount of radioactivity in the faeces was associated with malathion metabolic products due to phosphate action. The metabolic rate and products associated with various tissues were also studied and their importance discussed.

1. INTRODUCTION

Work in our laboratory previously established the presence of enzyme systems in the desert locust for activation of malathion into a potent anticholinesterase [1] and for the degradation of malathion into non-toxic products [2]. The purpose of the present investigation was to elucidate the pattern of overall metabolism of malathion by adults of the desert locust S. gregaria Forskal in vivo. This is important, since it is known that two alternate types of pathway are available for the metabolism of malathion (phosphatase type and carboxyesterase type) in vivo. Moreover, studies with different insect species suggested that the per cent of malathion metabolized through these pathways differed significantly, both qualitatively and quantitatively from one species of insect to another [3, 4].

2. MATERIALS AND METHODS

Adults of the desert locust S. gregaria Forskal, obtained from a laboratory colony and maintained according to Mehrotra and Rao [5], were treated topically with 10 µl solution of 1% ^{32}P-labelled malathion in benzene. This dose of malathion gave 99% mortality at 8 hours [5]. The topical application of malathion was made on the mesonotum with the aid of an electrically operated micro-applicator (ISCO Model M) manufactured by the Instrumentation Specialities Co. Inc., Lincoln,

Nebraska. ^{32}P-labelled malathion was supplied by the Atomic Energy Establishment, Trombay, and was identical chromatographically to the known malathion. The toxicity of radioactive malathion was comparable to that of the standard pure material. Malathion used as standard was kindly supplied by Dr. Lallan Rai of the Cyanamid India Ltd., Bombay.

The topically treated insects were rinsed with acetone to remove the unabsorbed malathion remaining on their surface. Immediately after rinsing, the radioactivity in various parts was determined. Various organs were homogenized in 10% trichloracetic acid, and the degradation products of malathion – carboxyesterase and phosphatase – were extracted according to the method of Matsumura and Hogendijk [6]. Faeces collected at various times was extracted in the same manner as other tissues.

All radioactivity measurements were made with the help of a Geiger-Müller thin end window tube at a fixed geometry and corrections were made for the background.

3. RESULTS

3.1. Absorption and distribution of ^{32}P-labelled malathion

Data on the distribution of radioactivity in desert locusts treated with 100 μg of ^{32}P-labelled malathion are presented in Figs 1 and 2. These data represent the total radioactivity of malathion and its degradation products present at various times in various tissues. It is apparent (Fig. 1, curve (a)) that 19.8% of the applied radioactivity penetrated within the first six minutes and that the internal radioactivity steadily increased to 45.2, 47.6, and 65.6% of the total applied dose at 90, 150 and 270 minutes, respectively, after treatment. Rapid and early penetration of the insecticide confirmed the findings of Buerger and O'Brien [7]. During the observation period of 270 minutes, the insects excreted 24.5% of the total applied dose in faeces (Fig. 1, curve (b)). Little excretion of radioactivity occurred during the first 150 minutes, but thereafter it increased. The thorax (Fig. 1, curve (c)) had the highest amount of radioactivity at 90 minutes, then equilibrium was maintained. The rapid rate of penetration ended at about 90 minutes; thence the rate of excretion predominated. In Fig. 2 we see that distribution of radioactivity in the gut (including malphigian tubules, main organ responsible for excretion) was similar to that observed for faeces. In the gut, as in the faeces, there was little activity initially, but after 150 minutes the activity increased. The amount of radioactivity in faeces and gut after 270 minutes accounted for 38.7% of the total dose applied, which is about 58.6% of the total malathion absorbed. In the abdomen (without gut) the amount of radioactivity increased until 150 minutes, at which time the abdomen had 7.8% of the total applied dose, and then decreased, being only 5.8% of the total applied dose at 270 minutes. Thus, as the radioactivity increased in faeces and gut, there was a corresponding decrease in radioactivity in the abdomen.

Levels of malathion or its metabolites in the head (the site of brain and nervous activity) varied from 3.3 to 5.3% of the total applied dose at various times (Fig. 3, curve (a)). Calculations of the per cent of the

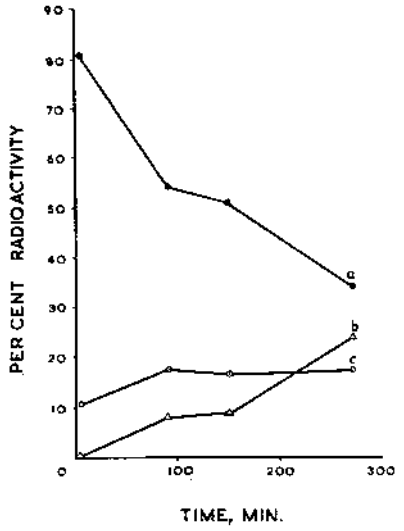

FIG. 1. Per cent recovery of radioactivity at different times from various parts of desert locusts topically treated with 100 μg ^{32}P-labelled malathion. (a) External wash. (b) Faeces. (c) Thorax.

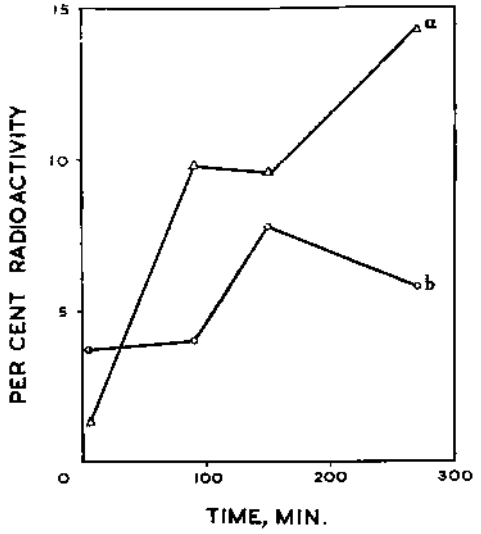

FIG. 2. Per cent recovery of radioactivity at different times from various parts of desert locusts topically treated with 100 μg of ^{32}P-labelled malathion. (a) Gut. (b) Abdomen.

total internal malathion gave values of 18.6, 14.2, 12.3 and 7.8% at 10, 90, 150 and 270 minutes, respectively (Fig. 3, curve (b)), suggesting that a large percentage of the initial internal dose of malathion found its way into the head (or brain) and this concentration of activity remained constant from 10 minutes onward. The constant level of radioactivity in the head indicated that when the head had attained equilibrium with the

internal levels of malathion the excess malathion was diverted to other organs, especially the gut.

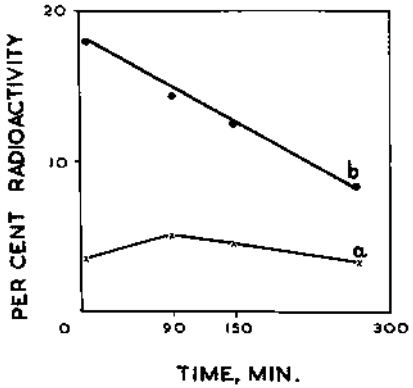

FIG. 8. Per cent recovery of radioactivity at different times from the head of desert locusts treated with 100 μg of ^{32}P-labelled malathion. (a) Per cent of total applied dose. (b) Per cent of internal activity present in the body.

3.2. In vivo metabolism of malathion in the desert locust

3.2.1. Metabolism in whole insects

Since metabolism of malathion can take place by two different pathways, namely (a) by carboxyesterase action, i.e. hydrolysis of COO C2 H5 bond of malathion, and (b) by phosphatase action, i.e. by hydrolysis of PSC bond of malathion, the metabolic products appearing in locusts and their various tissues were analysed to determine the predominant pathways present in the locust. The data (Table I) indicated that of the radioactivity initially present (0-6 minutes) inside the locust, carboxyesterase products predominated over phosphatase products. However, with the increase in time, the per cent of phosphatase products present in the body increased steadily. These results could be interpreted in two ways: (1) that the carboxyesterases were active in degrading malathion in initial stages when the overall low concentrations of chloroform-soluble (malathion/malaoxon) materials were present in the body. Later, however, with the increase in chloroform-soluble materials inside the body, carboxyesterases were inhibited and the main degradation route was shifted to phosphatase action. This view was supported by Matsumura and Hogendijk [6], who showed that carboxyesterases were inhibited by the high concentrations of oxygen analogues of malathion. (2) Since the malathion acid formed as a result of carboxyesterase action could be further metabolized by phosphatase action, it was likely that carboxyesterases were active throughout but their degradation products failed to accumulate because of further phosphatase action. The presence of significant amounts of carboxyesterase products at 270 minutes suggested that the second explanation was most probably correct. Nevertheless, the possibility that phosphatase action plays an important role in detoxication of malathion by the locust should also be kept in view.

The data in Table I indicated that the quantity of chloroform soluble products continued to increase up to 150 minutes, suggesting that the rate of penetration of the insecticide was faster than the rate of degradation and after 150 minutes, perhaps due to less penetration of the malathion, degradative action was more prominent.

3.2.2. Excretion of malathion and its metabolites

The data presented in Table II show that the major portion of the radioactivity in faeces was associated with the phosphatase products and only the remainder with carboxyesterase products. These findings are in agreement with the results obtained with whole insects, which revealed that phosphatase products predominated over the carboxyesterase products. However, the presence of significant amounts of chloroform-soluble metabolites in the faeces was rather surprising, since it is generally thought that in insects excretion is of little importance in disposing of lipid-soluble compounds [3].

Other researchers working with different insects showed that lipid-soluble materials, especially insecticides, were excreted by the insects. Bull et al. [8] showed that excreta of the larvae of the bollworm Heliothis zea contained large quantities of unchanged dimethoate as well as its oxygen analogues after being treated with dimethoate. Similarly, it has been shown that Heliothis zea larvae treated with ^{32}P-labelled disyston excreted a large percentage of radioactivity in faeces, and that 60 to 70% of the excreted radioactivity was due to chloroform-soluble compounds [9]. Gerolt [10] observed that house flies topically treated with dieldrin excreted nearly 11% of the total dose as hexane-soluble compounds. In contrast, the adults of the bollweevil, Anthonomas grandis, were not able to excrete the lipid-soluble and toxic products when treated with disyston [9]. Perhaps some species of insects are peculiar and are able to excrete lipid-soluble compounds; if so, the excretion of insecticides as such may be an important protective mechanism in some insect species.

3.2.3. Metabolism of malathion in various tissues

Since various tissues of the body have their own characteristic metabolic rate, the metabolic products associated with each tissue were analysed to determine the predominant pathway(s) in various tissues for malathion metabolism.

(a) Head

Since the data in Fig. 2 indicated that a large percentage of the internal malathion ^{32}P was in the head, we decided to determine the form in which it was present. This was important because the head contains the brain (main organ of nerve activity) and because most of the insecticides are known to be neurotoxic. Data presented in Fig. 4 showed that more than half of the radioactivity in the head was due to chloroform-soluble metabolites. The chloroform-soluble metabolites in the head at various times were found to be 2.21, 2.76, 2.40, and 1.98 μg equivalents of malathion at 0-6, 90, 150, and 270 minutes,

TABLE I. IN-VIVO METABOLISM OF MALATHION BY DESERT LOCUSTS TREATED WITH 100 μg OF ^{32}P-LABELLED MALATHION[a]

Time (min)	Percentage of the total applied dose recovered	Percentage internal radioactivity present as:		
		Chloroform-soluble products	Phosphatase products	Carboxyesterase products
0-6	19.8	69.9 (14.0)	9.7 (2.0)	21.9 (4.4)
90	33.0	64.4 (23.8)	21.5 (7.9)	14.5 (4.3)
150	39.3	65.2 (25.4)	23.7 (9.2)	11.0 (4.3)
270	41.1	36.4 (14.9)	35.1 (14.3)	28.4 (11.6)

Note: The figures in brackets represent μg equivalent of malathion present inside the body of the locust

(a) Average of two separate determinations; each datum represents 8 adult males of S. gregaria.

TABLE II. EXCRETION OF MALATHION BY DESERT LOCUSTS TREATED WITH 100 μg OF ^{32}P-LABELLED MALATHION[a]

Time (min)	Percentage recovery of the applied dose in faeces	Percentage radioactivity recovered as:		
		Chloroform-soluble products	Phosphatase products	Carboxyesterase products
0-6	0	0	0	0
90	8.2	4.2 (0.34)	73.1 (5.99)	22.6 (1.85)
150	8.3	24.7 (2.05)	56.3 (4.67)	16.0 (1.33)
270	24.5	36.2 (8.87)	55.9 (13.69)	7.8 (1.91)

Note: Figures in brackets represent the µg equivalent of ^{32}P-labelled malathion.

(a) Average of two separate determinations; each datum was obtained from the faeces collected from 8 adult males of S. gregaria

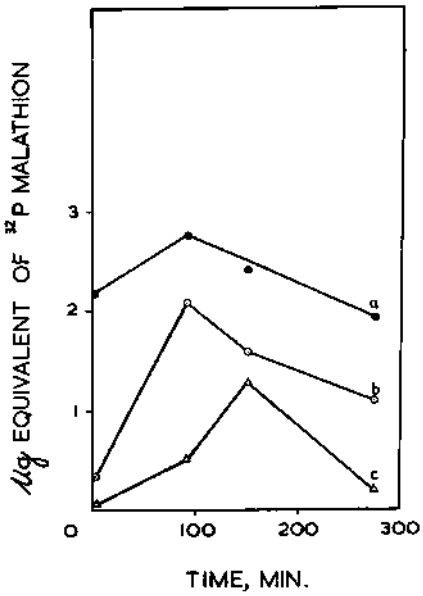

FIG. 4. Amounts of malathion and its metabolites present at various times in the head of desert locusts treated with 100 µg of ^{32}P-labelled malathion. (a) Chloroform-soluble products. (b) Products due to phosphatase action. (c) Products due to carboxyesterase action.

respectively. This suggested that at any time not more than 2.76 µg of malathion was present in the head. Since the average weight of the head was found to be 181 mg this meant that at any time there would not be more than 5.0×10^{-5} M concentration of malathion present in the head provided it was equally distributed throughout. The p I_{50} for malathion against insect cholinesterase was 4.6 [11], suggesting that no more than 20 to 30% of the head cholinesterase would be inhibited at that time. Cholinesterase inhibition in the head of a locust following treatment with 120 µg of malathion was observed to be 28% at one hour and 48% at six hours (Mehrotra, unpublished). Although no attempt was made to estimate the per cent of radioactivity due to malaoxon in chloroform-soluble metabolites, it was assumed negligible, since cholinesterase was inhibited more than that observed by Mehrotra (unpublished). These results confirmed the findings of Mehrotra and Sone Lal [1], who found that the head of Schistocerca gregaria was unable to activate malathion to any great extent. In the head, the radioactivity in the water-soluble metabolites of malathion was mainly due to phosphatase action and to a lesser extent to carboxyesterase action. High amounts of metabolites due to phosphatase action were seen at 90 and 150 minutes. Although the degradation products from carboxyesterase action were always present, the concentration of metabolites due to phosphatase action was more than that due to carboxyesterase action.

(b) Gut, abdomen and thorax

The distribution of radioactivity in various metabolites in gut, abdomen and thorax is given in Table III. In the gut the chloroform-

soluble metabolites continued to increase steadily up to 270 minutes at which time the gut contained 3.65 μg equivalent of malathion. On the other hand, in the abdomen (without gut) the high concentrations of chloroform-soluble material which were present initially decreased with time; the lowest amount (1.61 μg equivalent malathion) was present at 270 minutes. The total chloroform-soluble metabolites present in the gut and the abdomen, however, increased steadily from 0 to 270 minutes, suggesting that the gut took an active part in excreting the chloroform-soluble metabolites. Similarly, there was a continuous increase in metabolic products due to phosphatase action and carboxyesterase action in both gut and abdomen. Although the concentration of carboxyesterase products was high initially in all the three tissues, the ratio was nearly 2 : 1 for radioactivity in carboxyesterase products and phosphatase products, respectively. This trend reversed after 90 minutes. The main metabolic products were due to phosphatase action at 270 minutes and the ratio of radioactivity in carboxyesterase products versus phosphatase products approached 1 : 5. The chloroform-soluble metabolite in the thorax varied from 6-12% at various times.

4. DISCUSSION

Results reported in the present investigation lead to a few tentative conclusions. Firstly, there appeared to be two phases of the insecticide penetration through the cuticle after topical treatment, an initial rapid phase and a later slower phase. This biphasic permeability of the cuticle, not only in insects, but also in mammals, reptiles and amphibians, was recently recognized by Buerger and O'Brien [7]. The physiological reasons for such behaviour of the cuticle are still not fully understood.

Since the physiological action of the organophosphorous insecticides was supposed to be due to the inhibition of the enzyme cholinesterase in nervous tissue [12], the discovery that a small per cent (maximum 5.0 per cent) of the applied dose reached the head, the main seat of nervous activity, was significant. These results were similar to those reported by Fernando et al. [13], Benjamini et al. [14], Iyatomi et al [15] and Arthur and Casida [16], who observed that a small per cent of the total insecticide applied to the American cockroach reached the head, i.e. the nerve tissue. However, the amount of actual toxicant reaching the nervous tissue (or head) was different for different insecticides. Thus Fernando et al.[13] observed that 0.55% of 5.0 μg paraoxon reached the central nervous system of the cockroach, whereas 0.82% of TEPP and 0.24% of parathion were observed in the central nervous system 60 minutes after treatment with 5 μg of these insecticides. Our results obtained with the desert locust Schistocerca gregaria were different in that about 5% of the applied dose was found in the head. The difference in our results and those reported by Fernando et al.might be due to the high dose (100 μg/insect) in our experiments and the comparatively low dose (5 μg/g) used by Fernando et al.[13]. Moreover, their data referred to the insecticide in the nerve tissue only, whereas, in our experiments, the whole head, including other tissues, was taken. The differences observed may thus not be real ones.

TABLE III. IN-VIVO METABOLISM OF ^{32}P-LABELLED MALATHION IN THORAX, ABDOMEN AND GUT OF DESERT LOCUSTS TREATED WITH 100 μg OF MALATHION[a]

Time (min)	Percentage of total applied dose recovered	Per cent radioactivity recovered as:		
		Chloroform-soluble products	Phosphatase products	Carboxyesterase products
		Gut		
0-6	1.4	60.4 (0.9)	9.5 (0.1)	25.0 (0.4)
90	9.8	22.1 (2.3)	65.8 (6.5)	11.5 (1.1)
150	9.8	22.9 (2.3)	65.9 (6.5)	11.2 (1.1)
270	14.2	25.7 (3.5)	62.5 (8.4)	15.4 (2.1)
		Abdomen (without gut)		
0-6	3.6	65.5 (2.8)	13.4 (0.5)	21.0 (0.8)
90	4.0	41.3 (1.6)	43.1 (1.7)	15.5 (0.6)
150	7.8	25.6 (2.1)	64.3 (5.1)	10.0 (0.8)
270	5.8	27.7 (1.4)	63.1 (3.2)	9.1 (0.5)
		Thorax		
0-6	10.8	58.6 (6.0)	9.2 (1.0)	32.1 (3.2)
90	17.7	70.2 (12.6)	22.0 (3.9)	7.5 (1.4)
150	16.7	43.5 (7.0)	50.6 (8.0)	5.8 (1.0)
270	17.5	71.3 (12.1)	24.5 (4.3)	4.1 (0.8)

Note: Figures in brackets represent μg equivalent of malathion present in various tissues.

[a] Average of two separate determinations; each datum represents tissues obtained from 8 adult males of *S. gregaria*.

In conclusion, the following events took place when desert locusts were treated with a lethal dose of malathion. There was a rapid penetration of topically applied malathion and a large percentage of it was excreted in faeces. A small portion of the applied dose reached the head and fluctuated very little over a long period. Perhaps such a small quantity of malathion would be sufficient to disrupt the functioning of the nervous system.

ACKNOWLEDGEMENTS

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REFERENCES

[1] MEHROTRA, K.N., SONE LAL, Indian J. Ent. 29 (1967) 44.
[2] MEHROTRA, K.N., SETHI, G.R., BHAMBURKAR, M.W., ICAR Seminar on the Use of Radioisotopes and Radiations in Agriculture and Animal Husbandry (1968).
[3] O'BRIEN, R.D., Toxic Phosphorus Esters, Academic Press (1961).
[4] O'BRIEN, R.D., WOLFE, L., Radiation, Radioactivity and Insects, Academic Press (1964).
[5] MEHROTRA, K.N., SETHI, G.R., Indian J. Ent. 28 (1966) 114.
[5a] MEHROTRA, K.N., RAO, P.J., Indian J. exptl Biol. 4 (1966) 56.
[6] MATSUMURA, F., HOGENDIJK, C.J., Ent. exp. appl. 7 (1964) 179.
[7] BUERGER, A.A., O'BRIEN, R.D., J. cell. comp. Physiol. 66 (1965) 227.
[8] BULL, D.L., LINDQUIST, D.A., HAESKAYLO, J., J. econ. Ent. 56 (1963) 129.
[9] BULL, D.L., J. econ. Ent. 58 (1965) 249.
[10] GEROLT, P., J. econ. Ent. 58 (1965) 849.
[11] O'BRIEN, R.D., J. econ. Ent. 49 (1956) 484.
[12] O'BRIEN, R.D., A. Rev. Ent. 11 (1966) 369.
[13] FERNANDO, H.E., ROAN, C.C., KEARNS, C.W., Ann. ent. Soc. Am. 44 (1951) 551.
[14] BENJAMINI, E., METCALF, R.L., FUKUTO, T.R., J. econ. Ent. 52 (1959) 94.
[15] IYATOMI, K., SAITO, T., KANEHISA, K., NISHIZAWA, K., NARUSE, H., Botyu Kagaku 22 (1957) 192.
[16] ARTHUR, B.W., CASIDA, J.E., J. econ. Ent. 51 (1958) 49.

DISCUSSION

J.E. TREHERNE: First, I am interested in the biphasic penetration of malathion which you describe and which appears to parallel the situation observed in mammalian skin. Some years ago I demonstrated a reservoir effect in the penetration of a series of non-electrolytes through mammalian skin and erected a mathematical model to describe this phenomenon (J. Physiol. 131 (1956)). This model involved the postulation of a peripheral diffusion barrier which had the form of a lipid/water/lipid sandwich. It would be interesting to see whether the kinetics of malathion penetration through the cuticle could be accounted for by the equations derived for mammalian skin.

K.N. MEHROTRA: We ourselves were surprised at first at these fast rates of penetration. However, further investigations were carried out by one of our students, Miss Saroj Tule, who has studied this problem

rather extensively with a number of compounds; her results do not, I am afraid, follow the pattern of results you obtained with Schistocerca, published in the Journal of Insect Physiology (1957). I am not sure whether the model you postulate for mammalian skin would work for insect cuticle or not.

J.E. TREHERNE: Secondly, I am a little troubled by your assumption that the rate of appearance of radioactivity in the head is an accurate measure of the arrival of labelled compounds at the central nervous system. This does not, for example, take account of the arrival of labelled compounds at the ventral nerve cord or at the peripheral nerves. Furthermore, I think that it is highly desirable to express the concentration of toxic materials in terms of unit weight of nervous tissues rather than as total quantity in the head. This is especially important in view of the fact that the cerebral tissues contribute only a small fraction of the total weight of the head and also that interpretation of the mechanism of toxic action in neurophysiological terms requires a knowledge of the concentration of compounds within specific nervous tissues.

K.N. MEHROTRA: We did not look at the nervous tissues in the set of experiments reported here because of the difficulties involved in dissecting insects containing large quantities of radioactivity. However, I can assure you that we have since progressed much further and in later work (by Mehrotra, Phokela and Saasena, unpublished) the nervous tissue alone is considered.

W.J. LEQUESNE: Malathion labelled with sulphur-35, carbon-14 and tritium is also available commercially. Application of these isotopes would enable the insecticides to be located more accurately in the various organs by means of sectioning and autoradiography. Use of other isotopes, or even of double labelling, would perhaps make it possible to recognize the two halves of the molecule produced by detoxication as the result of phosphatase activity.

K.N. MEHROTRA: Certainly, one could use malathion labelled with ^{35}S, ^{14}C, or ^{3}H. Of these, ^{35}S would probably be unsatisfactory since it would give insufficient information, and tritium labelling, especially for malathion, may not be very useful because of the problem posed by exchange reaction, whereas ^{14}C has the advantage of having a long half-life and could conveniently be used to label different parts of the molecule. Autoradiography could, of course, be used, but I am afraid that the results would be qualitative rather than quantitative. I personally think that double labelling with ^{37}P and ^{14}C may be of real benefit, but in the last analysis it is a matter of experimental objective and of time, equipment and material available.

D

ISOTOPE APPLICATIONS: CHEMOSTERILANTS

(Session V, Part 2)

Chairman: L.K. CUTKOMP
UNITED STATES OF AMERICA

CHEMOSTERILANTS IN ENTOMOLOGY AND THE STERILE-MALE TECHNIQUE

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Abstract

CHEMOSTERILANTS IN ENTOMOLOGY AND THE STERILE-MALE TECHNIQUE. Chemosterilants have been often referred to as agents producing dominant lethal mutations in the germ cells of treated organisms. Recent studies of the mode of action of various chemosterilants in insects indicate that there are many other pathways by which chemical compounds can induce sterility in insects. Although the genetic mechanism still appears to be the most plausible explanation of the mode of action of many chemosterilants in male insects, other alternatives must be considered for explaining the chemical sterilization of female insects. The elucidation of the biochemical processes leading to sterility is important not only because chemical mutagenesis was first discovered in an insect (Drosophila melanogaster); chemicals offer a flexible, and sometimes the only available, method of inducing sterility in economically important insects which can be utilized in the sterile-male technique of insect control. As distinct from radiation, chemosterilants can be utilized for sterilization of the naturally occurring pest populations. In sterile-insect release programmes, chemosterilants become of importance whenever secondary radiation effects interfere with longevity and sexual competitiveness of radiation-sterilized insects. The understanding of the mechanism and mode of action of chemosterilants will aid in the search for new types of effective compounds and in designing new materials specific to insects or even to individual insect species. The sterility principle in pest control has thus far been successfully applied only to insects, but vertebrate pests will certainly be considered in the future. Chemosterilants may be the most effective tool for sterilization of vertebrate organisms.

By definition, chemosterilants are chemical compounds which reduce or destroy the reproductive capacity of an organism to which they are administered. In sexually reproducing organisms two main pathways of induced sterility may be considered: interference with the formation of a zygote and interference with the development of a zygote. A zygote cannot be formed if the male or the female doesn't produce a functional gamete or if the two gametes are prevented from combining. The death of a zygote can result from faulty genetic information imparted by either or both of the gametes or from environmental conditions which do not allow normal development.

Because insect chemosterilants were often referred to as being mutagenic I would like to discuss first which of the interferences which chemosterilants produce or may produce in insects are mutations in the accepted sense of the word. Unfortunately there doesn't seem to be a universal agreement on what mutation is. That mutation is a hereditary change in certain cellular components appears to be generally accepted but there is some controversy whether the cellular component is the nucleus, a gene, or simply a molecule of nucleic acid. With reference to individual insects the key term hereditary may refer either to the transmission of such changes from the parent (male or female) to the offspring or, within the insect's organism,

to the transmission from a parent cell to daughter cells. Dominant lethal mutations which are of particular importance in sterilization imply that the offspring die before reaching maturity or that the daughter cell ceases to be functional. With this concept in mind we can analyze some of the most important effects of chemosterilants in sexually reproducing insects.

Effect on males.

Aspermia and the lack of spermatozoan motility are seldom encountered in chemosterilized male insects. The most common effect of male chemosterilants is a condition of the sperm which for lack of better expression may be designated as sterility of the mature spermatozoa. The gametes of treated males appear to be in all respects normal [8] but the zygotes which they form die before reaching maturity. This effect corresponds to the classical definition of induction of dominant lethal mutations in the sperm and all of the known male insect chemosterilants which were examined in detail can be designated as mutagenic agents in the appropriate species of insects.

Effects on females

The effects of chemosterilants in female insects are variable, and they will be subdivided into several categories.

Treated females do not produce eggs

This type of sterility is usually a result of cytological damage to the reproductive organs, and it cannot be called mutagenic in the same sense as the male sterility mentioned earlier. Whether dominant or other lethal mutations were introduced into any cells of the female organism cannot be decided on the basis of inhibition of oogenesis or oviposition. Nutritional and hormonal factors are known to regulate the functions of the female reproductive system, and if the chemosterilant affects in some nongenetic way these factors its effects cannot be properly called mutagenic. Experimental evidence of such indirect activity of chemosterilants in female mosquitoes was obtained by Akov [1, 2]. Antimetabolites and alkylating agents were found to inhibit the digestion and elimination of blood which is a nutritional requirement for egg development in mosquitoes. Although Akov didn't prove or claim that the mechanism of sterilizing activity was nongenetic the evidence she presented suggests strongly that the primary reason for the sterility in female mosquitoes treated with the chemosterilants was a nutritional deficiency.

Treated females produce eggs which do not hatch

This type of activity is often only a function of the concentration or dose of the chemosterilant administered to the insect [6]. Again, the decision whether or not the mechanism of the sterilization was mutagenesis cannot be made without a detailed analysis of the primary and secondary effects of the chemical in the organism. If in eggs laid by treated females signs of incipient development are evident but the eggs fail to hatch the induction of dominant lethal

mutations must be certainly suspected. In absence of such evidence the compound cannot be termed mutagenic with respect to the whole organism but it still may be mutagenic with respect to some dividing cells of the organism.

Treated females produce eggs which hatch but the larvae die before pupating

This type of activity has been observed only in special classes of chemosterilants, particularly in the derivatives of s-triazine [7]. With respect to the treated insect the death of immature progeny is a clear sign of dominant lethal mutations although the period between the administration of the chemical and the appearance of its effects may be quite long.

In this brief survey of effects of chemosterilants on insects I have tried to emphasize the point that chemical sterilization cannot be simply equated to mutagenesis without a detailed study of the mechanism by which the chemosterilant acts.

A second point which may be even more important in practice is the distinction between the primary and secondary effects of the chemosterilant. In this connection primary effects are those which the chemosterilant itself (compound A) exerts on the cellular constituents of the organism; secondary effects are those which are exerted by a different material (compound B) which is a derivative or metabolite of the chemosterilant A. If, for example, the insect organism converts the compound A to the compound B and if only the compound B functions as a sterilizing agent, the activity of compound A in other organisms will depend on the ability of those organisms to convert effectively A to B. It is improbable that chemosterilants with a broad spectrum of activity would function via such secondary effects. Indications are, however, that the highly specific chemosterilants, particularly the nonalkylating phosphoramides and triazines, do indeed depend on the ability of the treated organism to convert them to effective, perhaps alkylating chemosterilants. Our current work on the mechanism of action of effective sterilants hempa and hemel [9] indicates that in house flies these dimethylamino compounds are selectively demethylated with the evolution of formaldehyde. Other analogous dimethylamino or methylamino compounds which are not demethylated in the same organisms are inactive as chemosterilants. Conclusive evidence supporting the hypothesis of secondary action is not yet available but the possibility of discovering chemosterilants which would be specific to only some organisms and relatively harmless to others is most attractive and deserves further investigation.

The third point which I wish to discuss is the application of chemosterilants. The role which this type of compound played in the history of genetics is well known although the early reports made references to chemical mutagens and not to chemosterilants. Nevertheless, the first report on chemical mutagenesis published by Auerbach and Robson [3] mentioned lethal mutations induced in Drosophila melanogaster with allyl isothiocyanate and with mustard gas [4]. In the Soviet Union, Rapoport described the mutagenic and

sterilizing effects of aldehydes [12] and carbamates [13] in 1946 and 1947. However, in genetic literature sterilizing effects were considered only in passing because the main interest was centered on the genetic makeup of the progeny of treated organisms. Organized effort directed toward the discovery of effective male and female insect chemosterilants started only in 1960 in the U.S. Department of Agriculture [5]. The main purpose of this search was to find and to develop an alternate method of sterilization of insects with radiation.

As compared to radiation, chemicals offer both advantages and disadvantages. Many species of insects which are killed by radiation before they are sterilized may still be sterilized chemically without undue mortality. Naturally occurring insects cannot be practically sterilized with radiation even if they are susceptible to it. Thus far chemosterilants appear to be the only method for sterilizing insects in natural populations.

On the negative side, chemosterilants share one of their most vexing problems with other chemical pesticides: whenever chemical compounds are used in nature the residue problem arises. High selectivity or species specificity may ease and minimize the difficulties but specificity is a two-edged sword. The more specific a chemosterilant is the more difficult, time-consuming, and costly it is to find it.

In conclusion I would like to mention one aspect of chemosterilants which has remained for all practical purposes unexplored. The effectiveness of the sterility principle in pest control depends on our ability to convert a population of largely fertile individuals to a population consisting largely of sterile individuals. The faster we can achieve this conversion the more effective will be the method. The main proponent of this method, E. F. Knipling [11], pointed out repeatedly that vertebrate populations are potentially as susceptible to being controlled by sterility as are the invertebrate populations. The question remains how to sterilize vertebrate organisms effectively and permanently. Radiation sterilization offers only very limited promise primarily because the vertebrate organism consists of too many systems and organs which are at least as sensitive to radiation as are the gonads. A release of artificially reared, sterilized animals cannot be considered in most instances and thus the sterilization of the natural population remains as the only practical possibility. Chemosterilants can be highly selective and specific even in higher organisms [10] but of course the problems of suitable treatment and of residues would require much research.

Whatever the ultimate application of chemosterilants will be, the basic research on the chemical interference with reproduction can stand on its own merit. Entomology has already profited from the intensified investigations of insect reproduction and mating during the past 10 years. The hormonal control and regulation of reproductory processes in insects are now only suspected or vaguely understood. There is little doubt that the research on chemosterilants will contribute to the knowledge of insect reproduction in the years to come.

REFERENCES

[1] AKOV, S., Inhibition of blood digestion and oocyte growth in Aedes aegypti by 5-fluorouracil, Biol. Bull. 129 3 (1965) 439.
[2] AKOV, S., Retention of the blood-meal in Aedes aegypti following sterilization of chemicals and irradiation, Ann. Trop. Med. Parasitol. 60 4 (1966) 482.
[3] AUERBACH, C., ROBSON, J.M., The production of mutations by allyl isothiocyanate, Nature 154 3898 (1944) 81.
[4] AUERBACH, C., ROBSON, J. M., Chemical production of mutations, Nature 157 3984 (1946) 302.
[5] BOŘKOVEC, A. B., Sexual sterilization of insects by chemicals, Science 137 3535 (1962) 1034.
[6] BOŘKOVEC, A. B., Insect Chemosterilants, Interscience, New York (1966).
[7] BOŘKOVEC, A. B., DEMILO, A. B., Insect chemosterilants. V. Derivatives of melamine, J. Med. Chem. 10 (1967) 457.
[8] CHANG, S. C., BOŘKOVEC, A. B., WOODS, C. W., Fate of tepa uniformly labeled with C-14 in male house flies, J. Econ. Entomol. 59 4 (1966) 943.
[9] CHANG, S. C., TERRY, P. H., BOŘKOVEC, A. B., Insect chemosterilants with low toxicity for mammals, Science 144 3614 (1964) 57.
[10] JACKSON, H., Antifertility Compounds in the Male and Female, Thomas, Springfield, Ill. (1966).
[11] KNIPLING, E. F., Sterile-male method of population control, Science 130 3380 (1959) 902.
[12] RAPOPORT, I. A., Carbonyl compounds and the mechanism of chemical mutagenesis, Doklady Akad. Nauk USSR 54 (1946) 65.
[13] RAPOPORT, I. A., Derivatives of carbamic acid and mutations, Byull. Eksp. Biol. Med. 23 (1947) 198.

DISCUSSION

C. A. PELERENTS: The results obtained in our laboratory with various products (Apholate, Metepa and Tepa) on Hylemya brassicae show that the differing degrees of sterility (failure to oviposit; oviposition without hatching of the eggs; hatching with subsequent death of the larvae) are a function of the applied dose. Is this a chance effect or is it commonly found?

A. B. BOŘKOVEC: This is a typical dose-effect relationship.

E. HORBER: I do not quite agree with your conclusions on the sterilization of insects in natural populations. We are not able to rear cockchafers (Melolontha sp.) in large quantities within a reasonably short time so our technique is to collect individuals from a natural population in an area where they are abundant and release them after irradiation in a region where the population has to be reduced or eradicated.

A. B. BOŘKOVEC: I should perhaps clarify my statement. When I referred to insects in natural populations I meant only those insects which, at the time of treatment, are part of a natural population. In contradistinction to this, your technique utilizes insects of natural origin which, at the time of treatment, are not part of a natural population. I do not, however, entirely exclude the possibility of achieving non-chemical sterilization of naturally occurring insects in the future.

E. HORBER: In the short time we have been working with Bombus sp. we noted two cases of sterilizing effects through parasitism in several of these social insects. One was caused by a nematode (Sphaerularia bombi) infecting the overwintering queen bumblebees individually. Another case was the result of several cockoobumblebee species (Tsithyrus sp.) occupying bumblebee colonies together after the worker brood had become available. I assume that naturally occurring sterilization is quite frequent among insects and would therefore suggest that the possibility be considered of analysing such cases for potential chemosterilants which might be more specific for insects and less harmful to vertebrates than the ones known hitherto.

A. B. BOŘKOVEC: This is an interesting phenomenon and it would indeed be worthwhile knowing whether sterility was caused by physical damage to the gonads or by some other, possibly chemical, process.

Rachel GALUN: With reference to Dr. Akov's work, (Refs [1] and [2] of the paper), I think one should distinguish between the effect of Fluoromacil and Apholate. While Apholate sterilizes both male and female mosquitoes, Fluoromacil affects only the females, by interfering with synthesis of the proteases necessary for blood digestion. In females treated with Apholate, retention of the blood meal is a secondary effect, due to inhibition of ovarian development.

T. R. ODHIAMBO: At what level, Dr. Bořkovec, did you carry out your morphological studies of the sperm from treated males? Did you, for example, examine them with the electron microscope? You stated that sperm from chemosterilized male insects appears to be normal in all respects. Could you give further information about your work on this aspect of the problem?

A. B. BOŘKOVEC: Two basic properties of sperm were considered: microscopic appearance and behaviour, that is, gross motility and ability to form a zygote. However, the work we did was very superficial and hence rather inadequate. It included examination of electron micrographs of treated and untreated house-fly sperm, but this revealed no obvious differences.

T. R. ODHIAMBO: Have you found any differences in the accessory reproductive glands in the treated male insects?

A. B. BOŘKOVEC: We have not gone into this aspect of the subject and, in fact, I know of no published work on the morphology of gonads of chemosterilized male insects.

T. R. ODHIAMBO: Could you suggest the mechanism which brings about death of the zygotes after injection of sperm from treated males?

A. B. BOŘKOVEC: I could put forward only those that are commonly offered to explain zygotic mortality caused by dominant lethals. Unfortunately, none of them fit our experimental data.

C. F. CURTIS: As regards possible damage to sperm caused by chemosterilants, it seems to me that the only question of practical importance is whether in a female mated to both a chemosterilized and a normal male, where the two types of sperm are thus in direct competition, equal numbers of viable and inviable zygotes are produced. Has this point been investigated?

A. B. BOŘKOVEC: This question can be answered only with reference to those insects where the female mates more than once. Scattered data in the literature and unpublished reports indicate that in fe-

males of some species the sperm from treated males can compete successfully with the sperm of untreated males. In other insects, the sequence of mating is the determining factor and, in still other instances, the sperm from treated males does not compete favourably with the sperm from untreated males. Such a lack of generality is typical in analyses of the effects of chemosterilants.

F. BERAN: Have you ever tried out the effect of triazine compounds, as used in weed control?

A. B. BOŘKOVEC: Yes, we have tested the effect on horse flies of many of the important herbicides based on s-triazine (see A. B. Bořkovec, G. C. La Breque and A. B. De Milo, J. econ. Ent. 60 (1967)). Most of these compounds showed a weak sterilizing activity but none was highly effective.

D. T. NORTH: As for the 'normal' appearance and behaviour of chemosterilant-treated sperm, I should like to mention some research done by Dr. La Chance at our Metabolism and Radiation Research Laboratory at Fargo, North Dakota, USA. Using sperm from Habrobracon, he has shown that treatment with the chemosterilant, Tepa, induces both dominant lethal mutations and sperm inactivation. Sperm inactivation occurs at relatively high doses, as compared with induced dominant lethals, and appears to be somewhat agent-specific. Dr. La Chance found that, whilst the phosphine oxide compounds, such as Tepa, cause sperm inactivation, triazine compounds appear to bring about only dominant lethals. It should be borne in mind that use of sperm inactivation as a type of male sterility could be of real practical value.

A. B. BOŘKOVEC: Sperm inactivation as observed in Habrobracon may be a condition of the sperm which does not allow formation of a zygote. On the other hand, it may not be related to any interaction between sterilant and sperm, but rather to an interaction between sterilant and seminal fluid. The expression 'sperm inactivation' is rather vague and is defined only in terms of the proportion of unfertilized eggs. It is conceivable that sperm itself is not even involved in such a process. I know of no chemical which would cause sperm inactivation only.

R. C. VON BORSTEL: Would the effects you ascribe to chemically induced inactivation of sperm apply to radiation-induced inactivation as well?

A. B. BOŘKOVEC: Yes, I think that radiation as well as chemicals could produce changes in the seminal fluid which would then interfere with fertilization.

V. DELUCCHI: It was mentioned that one of the main limitations on the application of chemosterilants in the field was the residue problem. I would appreciate having your personal opinion on the feasibility of using chemosterilants in the near future.

A. B. BOŘKOVEC: With certain reservations I should expect chemosterilants to be put to most immediate use in rearing-sterilization-release programmes, perhaps within the next 5 years. Other uses may follow if the safety problems can be solved sufficiently rapidly.

Rachel GALUN: Do chemosterilants decompose fast enough for it to be feasible to treat mass-reared insects and release them only after decomposition of the chemosterilant, so that any possible hazard is obviated?

A. B. BOŘKOVEC: Some chemosterilants do decompose at a sufficiently rapid rate, but the residue problem is a constantly recurring one,

which has to be solved for each individual compound applied to each given species.

A. LEMMA: It has recently been discovered in Ethiopia that at sub-lethal doses some molluscicides induce sterility in certain snails which transmit bilharzia. Do you know of any research done in this direction by other workers?

A. B. BOŘKOVEC: Not in connection with molluscs, but the effects of chemosterilants are not restricted to insects and chemosterilization of many other animals has certainly been described.

J. E. SIMON: I should like to call on Dr. Bořkovec's knowledge of South America to ask him whether he thinks that chemical sterilants should be used there or whether we should advise our young and often still inexperienced entomologists to wait until more is known of the effects of such compounds.

A. B. BOŘKOVEC: Chemosterilants should never be used unless suitable precautions are taken to ensure the safety of the operation. If chemosterilants are used outside the laboratory, that is, against the natural insect populations in the field, extreme precautions are necessary and such experiments perhaps should not be conducted when only inexperienced personnel are available. On the other hand, release of laboratory-sterilized insects does not involve handling dangerous chemicals in the field and your attention might thus more profitably be centred on such approaches to the sterility control method until more is known about the toxicology of chemosterilants. In the laboratory, of course, the use of chemosterilants does not present any problems different from those normally encountered in handling toxic chemicals.

H. ERDMAN: Until the mechanism of the sterilizing action of chemosterilants is known, these chemicals should in fact be treated with the same caution and respect as radioisotopes. The carcinogenic effect of some chemosterilants must prohibit their widespread use in natural environments.

R. C. VON BORSTEL: I should like to emphasize the importance of this comment with respect to the detectability of such compounds. Techniques are well worked out for detecting radioisotopes that have been released into the environment but the same is not true for alkylating agents though, in general, these induce similar cellular lesions. It is hardly desirable to take the occurrence of a skin lesion or a malignant tumour as criterion of the presence of an alkylating agent. I am sure Dr. Bořkovec would agree with this.

A. B. BOŘKOVEC: I certainly agree. All toxic chemicals should be handled with the utmost care. The operator should never judge the safety of his procedure from the fact that he is still alive.

M. COHEN: In raising the question of the potential importance of residues of chemosterilants one is drawing on the precedent of residue problems resulting from the use of pesticides. Many of the latter problems can be ascribed to misuse by farmers although some, of course, are due to use by contractors and Government agencies.

I suggest that chemosterilants would probably be administered solely by a central agency aiming at eradication in a particular area or region, rather than by individual farms. I should thus imagine that Governments would proceed with great caution in view of past experience with the pesticide residue problem.

ACTION DU TEPA SUR LE DEVELOPPEMENT NYMPHAL DE Dacus oleæ Gmel. (DIPTERA: TEPHRITIDAE)

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(Présenté par M. E. Tzanakakis)

Abstract — Résumé

ACTION OF TEPA ON THE PUPAL DEVELOPMENT OF Dacus oleæ Gmel. (DIPTERA: TEPHRITIDAE). Third-stage (fully grown) larvæ obtained from an artificial culture of Dacus oleæ were dipped, a few hours prior to pupation, into aqueous solutions of Tepa for 30 minutes. The following results were observed by the authors: even at the highest concentration tried (2%), Tepa had no effect on the mechanism of pupation, perhaps because of the late treatment; pupal development was inhibited after the larvæ had been dipped in solutions of above 0.125%, and death occurred at different stages of pupal life, the time of occurrence depending on the concentration of Tepa in the dipping solution; there was practically no difference between the minimum lethal dose and the maximum sterilizing dose, the safety factor being almost equal to 1. The authors discuss these results in comparison with those of a previous experiment during which Tepa was applied to adults shortly after they had emerged. The safety factor was then of the order of 50. In addition, the results of the two experiments mentioned above are compared with those of other experiments by different authors on the same insect subjected to gamma radiation. This comparison shows that, irrespective of the means of treatment (gamma rays or Tepa), the safety factor is large when the insects, at the time of treatment, are past the stage of histolysis-histogenesis.

ACTION DU TEPA SUR LE DEVELOPPEMENT NYMPHAL DE Dacus oleæ Gmel. (DIPTERA: TEPHRITIDAE). Des larves du troisième stade (stade larvaire complété), issues d'un élevage artificiel, furent immergées quelques heures avant la pupaison dans des solutions aqueuses de tepa, pendant 30 min. Les auteurs ont fait les constatations suivantes: le tepa, même à la plus forte des concentrations utilisées (2%), n'a pas eu d'effet sur le mécanisme de la pupaison, peut-être à cause de l'intervention tardive; le développement nymphal fut inhibé après l'immersion des larves dans les solutions utilisées à des concentrations supérieures à 0,125%, la mort est survenue à différents stades nymphaux, le temps de son apparition étant fonction des concentrations de tepa dans les solutions d'immersion; il n'y a pratiquement pas de marge entre la dose létale minimale et la dose stérilisante maximale, le facteur de sûreté (safety factor) étant presque égal à l'unité. Les auteurs commentent ces résultats et les comparent à ceux obtenus lors d'une expérience précédente au cours de laquelle le tepa était administré aux adultes peu après leur émergence. Le facteur de sûreté était alors de l'ordre de 50. En outre, les résultats de deux expériences sont comparés à ceux obtenus par d'autres chercheurs sur le même insecte après irradiation aux rayons gamma. De cette comparaison il résulte que, indépendamment du moyen de traitement (rayons gamma ou tepa), le facteur de sûreté est élevé lorsque les insectes, au moment du traitement, ont largement dépassé le stade d'histolyse-histogénèse.

INTRODUCTION

Au cours d'une expérience précédente [1], nous avons pu constater que le tepa, administré aux adultes de Dacus oleæ, avait une double action stérilisante sur leur système reproducteur: d'une part, il provoquait des dégâts au matériel génétique des œufs et des spermatozoaires, et, d'autre part, il inhibait le développement ovarien; ces deux modes d'action correspondaient à deux différentes zones de concentration du chimiostérilisant. Enfin le tepa, à une troisième zone de concentration, provoquait des

dégâts assez graves pour entraîner la mort des adultes traités. Cette troisième zone était assez éloignée des deux autres: le facteur de sûreté s'élevait à 50 pour la première zone (doses provoquant des dégâts au matériel génétique) et à 2 pour la deuxième (doses provoquant l'inhibition du développement ovarien).

Les substances chimiostérilisantes ayant dans leur molécule des groupes d'aziridinyl ont une action sur les acides nucléiques, et en particulier les ADN [2 - 4], qui sont responsables du développement et des diverses fonctions de la cellule; la mitose des cellules est inhibée et, par conséquent, le développement des tissus et des organes est bloqué. Les mêmes auteurs signalent que l'action des chimiostérilisants est plus forte lorsqu'il s'agit de tissus en pleine et rapide évolution.

Chez Dacus adulte, les organes en pleine évolution sont ceux du système reproducteur, d'où la grande sensibilité de ces organes à l'action cytotoxique des chimiostérilisants. A l'encontre des imagos, les pupes de Dacus, comme celles de tous les insectes holométaboles, subissent l'histolyse et l'histogénèse des tissus de la plupart de leurs organes; le système musculaire et l'exosquelette, par exemple, subissent des transformations beaucoup plus importantes que les organes reproducteurs.

Nous avons cru opportun d'examiner le cas d'une intervention au tepa au moment où les individus de Dacus oleæ n'ont pas encore atteint le stade de l'histolyse-histogénèse et où l'action cytotoxique du tepa sur divers systèmes et sur l'insecte entier peut être plus grande qu'au stade adulte.

MATERIEL ET METHODE UTILISES

Les insectes utilisés provenaient de l'élevage artificiel de notre laboratoire et étaient alimentés au stade larvaire avec de la nourriture préparée selon la formule de Tzanakakis et Economopoulos (type M) [5]. Les larves du troisième stade, peu après leur sortie à la surface supérieure de leur milieu nutritif, étaient transportées dans une fiole d'Erlenmeyer contenant une solution aqueuse de tepa[1]. Après l'immersion, elles étaient égouttées au moyen d'un papier filtre et placées dans des boîtes de Pétri. Les larves dont la pupaison ne s'effectuait pas dans les 5 heures qui suivaient l'immersion étaient rejetées.

La sortie des adultes survint entre le 10^{e} et le 14^{e} jour après la pupaison. Le 20^{e} jour, les pupes n'ayant pas donné lieu à des émergences d'adultes furent disséquées et examinées au binoculaire; les pupes mortes furent ensuite classées dans l'une des trois catégories suivantes: a) pupes mortes avant ou pendant le stade de l'histolyse-histogénèse; b) pupes mortes après la formation d'adultes mais avant la pigmentation de leur cuticule; seuls les yeux avaient une coloration rose-violette pâle; les ébauches des ailes étaient apparentes; c) pupes mortes après la pigmentation de la cuticule des adultes; les adultes paraissaient complètement formés; quelques-uns de ces adultes étaient encore vivants au moment de la dissection, mais non viables.

[1] Après plusieurs essais préliminaires sur les concentrations du chimiostérilisant et la durée d'immersion convenables pour l'expérience, nous avons utilisé en définitive des concentrations de 2, 1, 0,5, 0,25 et 0,125% et une durée d'immersion de 30 min. Des larves immergées dans de l'eau pendant 30 min ont été prises comme témoins.

Lorsqu'il y eut émergence d'adultes, ceux-ci furent transportés dans des cages de 30 × 30 × 30 cm (20 couples par cage et deux répétitions par concentration de la solution d'immersion). La nourriture était constituée par de la levure hydrolysée, du sucre et de l'eau à raison de 1 : 4 : 5. Après le 10e jour, des fruits artificiels de paraffine étaient placés dans les cages pendant 24 heures; les œufs pondus étaient ensuite transportés dans des boîtes de Pétri pour le contrôle du pourcentage d'éclosion des œufs. Trente deux jours après l'émergence, les femelles étaient disséquées et leurs ovaires examinés au binoculaire pour le contrôle de l'action cytotoxique du tepa; en même temps, l'examen des spermathèques s'effectuait au microscope pour le contrôle de la vigueur sexuelle et de l'état des spermatozoaires (mobilité, forme).

La température du laboratoire oscillait entre 23 et 26°C et l'humidité relative entre 48 et 63%. La lumière sur la surface en verre des cages était de 500 à 800 lux pour une photopériode de 12 heures.

RESULTATS

Les données expérimentales figurent dans les tableaux I, II et III. On peut constater que:

1) Le tepa, même à la concentration de 2,0%, n'a pas eu d'effet sur le mécanisme de la pupaison; le pourcentage de pupaison des larves immergées dans différentes solutions de tepa oscille entre 94 et 100% (tableau I).

2) Le développement nymphal a été inhibé après l'immersion des larves dans des solutions de tepa aux concentrations supérieures à 0,125% (tableau I).

3) La mort des pupes est survenue à différents stades nymphaux (tableau II). La vitesse d'apparition de la mort paraît être fonction de la concentration de tepa dans la solution d'immersion: la plus grande partie (79%) des larves immergées dans la solution à 2,0% n'a pas pu dépasser le stade de l'histolyse-histogénèse; par contre, aux concentrations de 0,5%

TABLEAU I. ACTION DU TEPA SUR LES PUPES DE Dacus oleæ APRES UNE IMMERSION DE 30 min DES LARVES (3e STADE) DANS UNE SOLUTION AQUEUSE DU CHIMIOSTERILISANT

Nombre de L_3 utilisées	Concentration du tepa (%)	Pupaison des L_3 (%)	Emergence des L (%)
82	2,0	93,8	0
81	1,0	100,0	1,4
103	0,5	100,0	2,9
97	0,25	100,0	48,5
99	0,125	100,0	78,3
110	0	100,0	81,3

TABLEAU II. STADES NYMPHAUX PENDANT LESQUELS EST SURVENUE LA MORT, APRES UNE IMMERSION DE 30 min DES LARVES DE Dacus oleæ (3e STADE) DANS UNE SOLUTION AQUEUSE DE TEPA

Nombre de L_3 utilisées	Concentration du tepa (%)	Pupes mortes (% des L_3)			Adultes morts pendant l'émergence (% des L_3)
		Avant ou pendant l'histolyse-histogénèse	Après la formation d'adultes et avant la pigmentation de leur cuticule	Après la pigmentation de la cuticule des adultes	
82	2,0	79,1	18,4	2,4	0
81	1,0	39,3	43,0	16,2	0
103	0,5	23,3	32,8	29,4	11,6
97	0,25	18,2	16,2	11,1	6,0
99	0,125	8,2	6,2	4,2	3,1
110	0	10,2	1,9	2,9	3,8

TABLEAU III. FECONDITE, FERTILITE ET VIGUEUR SEXUELLE DES ADULTES DE Dacus oleæ ISSUS DE LARVES IMMERGEES PENDANT 30 min DANS UNE SOLUTION AQUEUSE DE TEPA (40 couples d'adultes par concentration)

Concentration du tepa (%)	Nombre d'œufs pondus pendant 22 jours	Eclosion d'œufs (%)	Spermathèques fécondées (%)
0,25	1249	26,9	67,0
0,125	1123	79,1	85,0
0	986	82,3	90,0

et de 0,25%, la partie des pupes mortes avant ou pendant l'histolyse-histogénèse ne représentait pas même le tiers des pupes mortes.

4) Le pourcentage d'adultes morts pendant la sortie ne paraît pas dépendre de la concentration de tepa dans le liquide d'immersion (tableau II); le faible pourcentage observé chez les adultes issus des larves immergées dans les solutions de tepa, qui dépasse quelquefois celui des insectes témoins, ne représente en réalité que les adultes ayant des défauts qu'on rencontre bien souvent chez les témoins.

5) Les organes reproducteurs des femelles issues des larves immergées dans des solutions à 0,25% et 0,125% n'ont subi aucune action cytotoxique; le développement ovarien a été normal, comme l'a démontré l'examen microscopique 32 jours après l'émergence. D'autre part, la fécondité des femelles n'a pas été affectée par le tepa (tableau III).

6) Le pourcentage d'éclosion des œufs pondus par les femelles issues des larves immergées dans une solution à 0,25% s'est trouvé réduit à 26,9% (tableau III). On ne pourrait attribuer la réduction de la fertilité à la seule réduction du pourcentage des femelles fécondées (67,0%); une partie de femelles fécondées aurait pondu des œufs non viables.

DISCUSSION ET CONCLUSIONS

On peut conclure des résultats obtenus dans les conditions expérimentales de ce travail que le tepa a une action marquée aussi bien sur le système reproducteur que sur l'insecte entier lorsqu'il est administré avant que les insectes atteignent le stade de l'histolyse-histogénèse. A la concentration de 0,25%, les larves immergées pendant 30 min furent à la fois stérilisées et tuées (tableaux I et III); ainsi on ne peut pas distinguer les deux différents modes d'action du tepa, alors que, dans le cas de notre précédent travail [1], la mort des insectes ne survenait que lorsque nous utilisions des doses 50 fois plus fortes que celles qui provoquaient la stérilisation des adultes.

Tzanakakis et Thomou [6], en irradiant aux rayons gamma des pupes de Dacus oleæ âgées de 9 jours, provoquaient une stérilisation complète chez ces insectes à la dose de 6 krad; en établissant la courbe de toxicité, Tzanakakis [7] a trouvé qu'à la dose de 40 krad les rayons gamma ne

provoquaient qu' une faible mortalité lorsque les pupes irradiées étaient âgées de 9 jours. Dans les deux expériences, les pupes utilisées étaient à 1 ou 2 jours de l'émergence; les insectes avaient largement dépassé le stade de l'histolyse-histogénèse. En utilisant des larves de Dacus oleae un peu avant la pupaison et des pupes quelques heures après la pupaison, Fytizas et Tzanakakis (travail non publié) ont trouvé des résultats différents de ceux obtenus lorsque les pupes étaient âgées de 9 jours; ainsi, les larves et les pupes irradiées à 2 krad subirent l'action insecticide à 100% tandis que la totalité de celles qui avaient été irradiées à 1 krad échappèrent à la mort et qu'une partie d'entre elles échappèrent à la stérilisation; la dose létale minimale fut inférieure à 2 krad et la dose stérilisante maximale supérieure à 1 krad. Dans le cas des irradiations on ne peut pas non plus distinguer les deux actions (insecticide et stérilisante) lorsque les insectes traités n'on pas atteint ou dépassé le stade de l'histolyse-histogénèse.

Le facteur de sûreté (safety factor), rapport de la dose minimale létale à la dose maximale stérilisante, ne caractériserait pas seulement une substance comme chimiostérilisante ou insecticide; la même substance peut être considérée comme chimiostérilisante ou insecticide selon le mode d'application ou le temps d'intervention. Le tepa, par exemple, comme les rayons gamma, ne peut être considéré comme stérilisant lorsqu'il est administré à des larves ou de jeunes pupes de Dacus oleae.

En outre le tepa, aux doses de 0,25%, 0,5% et 1, 0%, a pu inhiber le développement nymphal d'une partie des insectes ayant dépassé le stade de l'histolyse-histogénèse; peut-être ce produit aurait-il aussi une action sur le mécanisme de l'émergence.

Enfin, l'absence d'action du tepa sur la pupaison pourrait être dû à l'intervention tardive, 2 à 5 heures avant la pupaison. Des travaux sur une action éventuelle sur la pupaison sont en cours, l'intervention étant effectuée à un stade plus précoce que dans ce travail.

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REFERENCES

[1] FYTIZAS, E., Action du Tepa sur les adultes de Dacus oleae Gmel. (Diptera: Tephritidae) en fonction des quantités du chimiostérilisant utilisées. Annls Inst. phytopath. Benaki (sous presse).

[2] BROOKS, P., LAWLEY, P.D., The reaction of mono- and difunctional alkylating agents with nucleic acids, Biochem. J. 80 (1961) 496-503.

[3] FAHMY, O.G., FAHMY, M.G., The chemistry and genetics of the alkylating chemosterilants, Trans. R. Soc. trop. Med. Hyg. 58 (1964) 318-26.

[4] FRAENKEL-CONRAT, H., Chemical modification of viral ribonucleic acid, I. Alkylating agents, Biochem. biophys. Acta 49 (1961) 169-80.

[5] TZANAKAKIS, M.E., ECONOMOPOULOS, A.P., Two efficient larval diets for the continuous rearing of the olive fruit fly, Dacus oleae (Gmelin) (Diptera: Tephritidae), J. econ. Ent. 60 3 (1967) 660-63.

[6] INTERNATIONAL ATOMIC ENERGY AGENCY, Advances in Insect Population Control by the Sterile-Male Technique, Technical Reports Series No.44, IAEA, Vienna (1965) 4.

[7] TZANAKAKIS, M.E., Control of the olive fruit fly, Dacus oleae (Gmelin), with radiation or chemical sterilization procedures. Technical Report, U.S.D.A., Grant FG-GR-102, Project EII-ENT-I (6 February 1967) 80pp.

DISCUSSION

A.B. BOŘKOVEC: What was the larval instar in which the insects were treated?

M.E. TZANAKAKIS: It was the end of the third and last larval stage. The fully grown larvae have then finished feeding, and leave the larval food medium in search of a more suitable place to pupate. Under the conditions in which the authors worked, formation of the puparium usually took place 2-5 hours later.

A.B. BOŘKOVEC: Do you know whether spermatogenesis in the male larvae was completed or in progress at the time of treatment with the chemosterilant?

M.E. TZANAKAKIS: I am afraid I do not have this information to hand, but I believe that spermatogenesis was still in progress at this stage.

E

STERILE-MALE TECHNIQUE

(Sessions VI and VII)

Chairman: E. SHUMAKOV

USSR

ТРУДНОСТИ И УСПЕХИ МАССОВОГО РАЗВЕДЕНИЯ НАСЕКОМЫХ В ЛАБОРАТОРИИ И ВОЗМОЖНОСТИ САМОИСТРЕБЛЕНИЯ НЕКОТОРЫХ ВРЕДНЫХ ВИДОВ

Е.М.ШУМАКОВ
ВСЕСОЮЗНЫЙ НАУЧНО-ИССЛЕДОВАТЕЛЬСКИЙ ИНСТИТУТ ЗАЩИТЫ РАСТЕНИЙ,
ЛЕНИНГРАД,
СССР

Abstract — Аннотация

DIFFICULTIES AND SUCCESSES IN THE MASS REARING OF INSECTS IN THE LABORATORY, AND THE POSSIBILITY OF AUTOCIDAL CONTROL OF SOME HARMFUL SPECIES. The practical development of the sterile-male release technique, as indeed of all methods of autocidal control of harmful insects, is limited by the difficulties of mass rearing in artificial conditions. However, analysis of cases of the successful solution of this problem for a number of types of Diptera, Lepidoptera and Orthoptera gives an indication of possible ways of setting up large-scale rearing of the insects required.

The most difficult problem, that of ensuring a suitable food supply for the insects at any time of the year, is being successfully overcome as a result of the progress made in producing synthetic and semi-synthetic nutrient media, which have been developed both for semi-saprophytic and for herbivorous and predacious species. The choice of recipes for such nutrients is determined by correct selection of the necessary ingredients, above all vitamins, free amino acids and sterols; the proper quantitative proportion between these substances and between the basic components of the food — proteins, fats and hydrocarbons — is of the greatest importance. The amount of work involved in insect rearing depends on what means are used for sterilizing the containers and for preventing the nutrient medium from decaying. One way in which these problems have been solved is by the development of a dried nutrient medium in powder form; this has been successfully used for rearing the migratory locust in the USSR. For a number of harmful species of Lepidoptera, it has been shown possible to rear the insects in a closed, sealed container. In this case a supply of nutrient medium is provided adequate to ensure the insects' development from the time the eggs or caterpillars are deposited until the imago of the new generation emerges. This approach greatly simplifies the whole process of mass rearing. Many species of insects can be reared on grain or plant seeds which are less subject to decay during prolonged storage.

Another important problem is that of developing ways of overcoming the diapause in laboratory populations in order to ensure continuous rearing. This can be done either by reactivating the insects by temperature changes or by instituting a period of illumination which prevents the diapause from starting. A further possible method is that of selecting and crossing diapausing and non-diapausing strains of a given species. A number of species of Orthoptera having a fairly wide natural habitat have been used to show the possibility of autocidal control by adding to a natural population which normally has a diapause specimens of a non-diapausing population from other parts of the habitat. This possibility has been demonstrated for the cricket Teleogryllus commodus Walk. in Australia and for sub-species of Locusta migratoria L. in the Old World. The development of this form of autocidal control of insects merits close attention.

The work reported is devoted mainly to developing methods of autocidal control and techniques for mass laboratory breeding of such harmful species as Carpocapsa pomonella L., Eurygaster integriceps Put. and Locusta migratoria L.

ТРУДНОСТИ И УСПЕХИ МАССОВОГО РАЗВЕДЕНИЯ НАСЕКОМЫХ В ЛАБОРАТОРИИ И ВОЗМОЖНОСТИ САМОИСТРЕБЛЕНИЯ НЕКОТОРЫХ ВРЕДНЫХ ВИДОВ. Практическая разработка метода выпуска стерилизованных самцов, как и вообще методов самоистребления вредных насекомых, лимитируется трудностями массового разведения их в искусственных условиях. Однако, анализ успешных случаев решения этой проблемы для ряда видов Diptera, Lepidoptera и Orthoptera позволяет наметить возможные пути создания техники массового разведения необходимых объектов.

Наиболее трудный вопрос обеспечения насекомых соответствующим кормом в любое время года успешно разрешается благодаря прогрессу, достигнутому в создании синтетических и полусинтетических пищевых сред для насекомых. Такие среды разработаны уже как для полусапрофитных, так и для растительноядных и хищных видов. Подбор рецептов для таких сред определяется правильным выбором необходимых компонентов, в первую очередь витаминов, свободных аминокислот и стеринов. Важнейшее значение имеет правильное количественное соотношение в пищевой среде как указанных веществ, так и основных компонентов корма – белков, жиров и углеводов. Трудоемкость процессов размножения насекомых определяется способами стерилизации посуды и осадков, а также методами предохранения пищевых сред от загнивания. Примером преодоления этих трудностей является создание сухой, порошкообразной среды для перелетной саранчи, на которой в СССР этот вид успешно воспитывается. Для ряда вредных видов Lepidoptera "показана возможность выращивания особей в закрытой, герметизированной посуде. При этом дается запас пищевой среды, обеспечивающий развитие особей с момента подсадки на среду яиц или гусениц до выхода имаго нового поколения. Такой прием весьма упрощает весь технологический процесс получения массовой продукции. Многие виды насекомых можно воспитывать на зерне и семенах растений, менее подверженных загниванию при длительном хранении.

Важнейшим вопросом массового разведения является разработка путей преодоления диапаузы у лабораторной популяции, что обеспечивает непрерывность разведения. Устранение диапаузы достигается как реактивацией температурными воздействиями, так и созданием фотопериода, предотвращающего возникновение диапаузы. Возможен еще один путь: подбор и скрещивание генетически различных рас данного вида, имеющих диапаузу и лишенных ее. На примере ряда видов Orthoptera, имеющих достаточно большие ареалы, показана возможность самоистребления вредных видов путем подмешивания к природной популяции, нормально имеющей диапаузу – особей бездиапаузной популяции из других частей ареала того же вида. Такие возможности выявлены для сверчка Teleogryllus commodus Walk в Австралии, и для подвидов Locusta migratoria L. на территории Старого Света. Разработка такого пути самоистребления насекомых заслуживает большого внимания.

В наших работах основное внимание уделяется разработке методов самоистребления и техники массового лабораторного разведения таких вредных видов как Carpocapsa pomonella L., Eurygaster integriceps Put. и Locusta migratoria L.

За последние десять лет во многих странах интенсивно развивались исследования в двух близких направлениях – разработка методов стерилизации насекомых и создание техники массового разведения их в лабораторных условиях. Хотя эти два направления возникли и развивались независимо и опирались на различные физиологические основы, очень скоро стала ясна органическая связь этих направлений.

Практические успехи применения стерилизации насекомых достигались лишь там, где была разработана заранее адекватная техника массового получения насекомых для их последующего облучения или обработки хемостерилянтами.

Развитие исследований в области химической стерилизации насекомых началось совсем недавно – лишь после 1960 года – но именно после этой даты особенно интенсивно стали разрабатываться искусственные пищевые среды для насекомых. Как показывает только что вышедший в свет обзор Хауса [1], например для Lepidoptera более 80% рецептов искусственных сред были опубликованы в работах, вышедших в свет после 1960 года. В работе Хауса совершенно не учтены исследования, проведенные в этом направлении в СССР, где первые публикации об искусственных средах также появились в 1960 году [2] и в последующие годы число их постепенно нарастало.

Таким образом, разработка методов воспитания насекомых на искусственной пище является очень молодой отраслью энтомологических исследований, особенно интенсивно разрабатывающейся лишь в последнее десятилетие. Это обстоятельство определяет ряд трудностей, не-

избежно возникающих перед исследователями, работающими во всякой новой отрасли науки. Вполне естественно, что теоретические (физиологические) основы разведения насекомых на искусственной пище еще не вполне разработаны, и хотя основанных на проведенных экспериментах обобщений высказано достаточно много, они отличаются значительной фрагментарностью и нуждаются в критическом анализе и экспериментальной проверке.

Вследствие молодости данного научного направления дает себя знать недостаток обобщения накопленных данных и небольшое количество свободных и обзорных работ по этому вопросу. Упомянутая выше работа [1] по существу является первым обзором, объединившим накопленные материалы по рецептуре искусственных сред для насекомых. Она охватывает преимущественно исследования, проведенные в США, Канаде, Великобритании и Японии, и показывает достижения в создании искусственной пищи для насекомых преимущественно из отрядов Coleoptera, Diptera, Lepidoptera и Orthoptera. Количество видов, для которых разработаны методы воспитания на синтетических средах в каждом из указанных отрядов, колеблется от 10 до 25, а всего Хаус рассматривает состав пищи примерно для 115 видов насекомых. В работе Хауса большой интерес представляет библиография обзорных работ по физиологии питания насекомых, данная в приложении. Эта библиография начинается с известного обзора Уварова [3] и заканчивается последними работами самого автора [4].

Хронологически работе Хауса предшествовали лишь обзорные работы по искусственным средам, опубликованные в СССР [5, 6].

В начале 60-х годов разведение насекомых-фитофагов на искусственных средах только начиналось. Но к этому времени уже были достигнуты значительные успехи в создании искусственных сред для насекомых-полусапрофагов (Drosophila melanogaster, Lucilia sericata, Cochliomya hominiv и др.), для видов питающихся зерном или мукой, а также пищевыми остатками (например, Tenebrio molitor, Tribolium confusum тараканы Blatta и Blatella и т.д.

Первые работы о разведении насекомых-фитофагов на искусственных средах появились лишь в 50-х годах и в начале опыта не приносили особых успехов, показав значительную трудность проблемы.

Лишь после того, как к синтетической среде были добавлены вещества растительного происхождения в виде размолотых сухих листьев или патентованного препарата из соков трав, известного под названием "церофил", были достигнуты первые успехи в воспитании полноценного потомства таких видов как Pyraustra/Ostrinia/nubilalis и Grapholita molesta (1949 – 1956 годы).

В последующие годы, однако, были найдены составы сред, без добавления каких-либо веществ растительного происхождения; на таких средах удалось выращивать ряд скрытоживущих насекомых, от которых было получено плодовитое потомство в течение нескольких поколений. К таким видам относятся Hylemyia antiqua, Pectinophora gossipiella, Pexicopia malvella, Chloridea obsoleta, Anthonomus grandis.

Воспитание насекомых, живущих скрыто, внутри растительного субстрата, удавалось легче, чем выращивание на синтетических средах фитофагов, живущих открыто на поверхности листьев.

Возможность воспитания на синтетических средах открытоживущих насекомых была показана нами [2] на примере гусениц Loxostege sticticalis, , а впоследствии и для некоторых других видов Lepidoptera.

За последующие 5 лет (1963 – 1967) количество работ, посвященных искусственным средам для различных как скрытоживущих, так и листоядных насекомых, прогрессивно возрастало во многих странах. При этом особое внимание уделялось технике получения массовой продукции насекомых, что стимулировалось потребностями разработки новых методов борьбы с вредителями, таких как "Sterile male technique" и других способов самоистребления вредных насекомых.

В связи с этим в США была предпринята попытка создать капитальное руководство по массовому разведению и колонизации насекомых [7], которая очевидно, в ближайшее время выйдет в свет и будет доступна заинтересованным исследователям.

Перечисленными выше работами практически исчерпываются обзорные труды по методике воспитания насекомых на искусственных средах.

Несмотря на молодость проблемы, следует отметить появление в самые последние годы некоторых теоретических обобщений, которые, вероятно, явятся полезными руководящими принципами в дальнейших исследованиях по разработке рецептуры искусственных питательных сред для насекомых.

Одним из таких важных теоретических исследований является другая работа Хауса [4], рассматривающая значение некоторых новых достижений физиологии питания насекомых для разработки проблем биологического метода борьбы с вредителями. Хаус устанавливает три принципа питания насекомых, которые он формулирует следующим образом:

1. Правило тождественности пищевых требований;
2. Принцип рационального соотношения пищевых компонентов;
3. Принцип компенсирующих добавок.

Эти обобщения исследований, проведенных канадскими энтомологами в Бельвилле, заслуживают серьезного внимания.

Исследования, проведенные в СССР, также позволили сделать некоторые обобщения. В наших работах [8] показаны пищевые различия насекомых, живущих внутри растительного субстрата (скрытоживущие) и обитающие открыто, питающиеся листвой растений. Выяснено, что для видов, развивающихся в природе на плодоэлементах, оптимальными являются среды с большим количеством азотистых веществ и жиров, но относительно бедных углеводами и стеринами. Для листоядных насекомых напротив, необходимы среды богатые углеводами и стеринами, в то время как относительный избыток жиров и азотистых веществ действет на этих насекомых угнетающе. Указанные различия пищевых требований насекомых этих двух типов являются отражением различий биохимического состава природной пищи этих двух групп фитофагов.

Важным обобщением советских исследователей является также выяснение роли свободных аминокислот, как необходимых компонентов пищи. Изучение питания тутового и дубового шелкопрядов (Bombyx mori и Antheraea pernyi), проведенное Филипповичем [9 – 12], показало, что выбор пищи в значительной мере определяется содержанием в ней определенного набора свободных аминокислот. В наших работах также показано, что даже у видов, развивающихся в природе на одном и том же растении (хлопчатнике), потребности в наборе свободных аминокислот

различны, что видно на примере сравнительного изучения пищевых требований Gelechia malvella и Chloridea obsoleta [6].

Приведенные выше обобщения стали возможны, главным образом, благодаря применению методов воспитания насекомых на синтетических пищевых средах, лишенных продуктов растительного происхождения. Эти исследования позволили далеко вперед продвинуть наши представления о роли отдельных компонентов пищи в насекомых и более обоснованно подбирать рецептуру искусственной пищи для массового разведения насекомых в лабораторных условиях.

Но пожалуй самым важным теоретическим достижением последних лет является отказ от представления о том, что выбор пищи насекомых зависит от наличия каких-либо специфических веществ, получивших название акцептантов, фагостимулянтов и т.д. Еще до 1956 года эти представления были очень распространены и защищались некоторыми авторами [13, 14]. Однако, по мере того, как были достигнуты успехи в разведении насекомых на чисто синтетических средах стало ясно, что привлекательность пищи зависит не от наличия в ней фагостимулянтов, а от соотношения и набора основных питательных веществ, которые являются общими для всех насекомых. Обязательными компонентами пищевых сред для всех насекомых являются азотистые вещества (белки и аминокислоты), липиды (жиры и стерины), углеводы, а также набор необходимых витаминов и минеральных солей.

Успех воспитания насекомых на синтетических средах зависит не только от химического состава сред, но и от их консистенции. Для многих скрытоживущих насекомых, например Pexicopia malvella или Carpocapsa pomonella, среда является не только пищевым субстратом, но и средой обитания, в которой гусеницы прокладывают свои ходы, строят камеру для окукления и т.д., поэтому она должна обладать соответствующей консистенцией. Для этого в среды добавляется агар, целлюлоза, а в некоторых случаях и альгиновая кислота, для связывания влаги. Консистенция среды в особенности должна учитываться при разведении открытоживущих насекомых. Так, например, в наших опытах особые трудности представил подбор пищевых сред для гусениц Porthetria dispar и эти трудности удалось преодолеть лишь после того, как в среду была добавлена альгиновая кислота, придавшая ей значительную плотность.

В качестве примера синтетических сред, применяемых в наших работах для разведения ряда видов Lepidoptera, в табл. 1 приводится следующий рецепт:

Указанный рецепт представляет среду, обладающую известной универсальностью, так как применяя некоторые вариации ее состава оказалось возможным воспитывать на такой среде следующие виды насекомых: Chloridea obsoleta, Ostrinia nubilalis, Pexicopia malvella, Loxostege sticticalis, Hadena sordida и Carpocapsa pomonella [8].

Чисто-синтетические среды, подобные приведенной выше, конечно, нельзя рассматривать как перспективные для массового разведения насекомых в промышленных масштабах, вследствие их сложности и дороговизны. Однако, такие среды служат исходным моментом для выяснения пищевых требований отдельных видов и для создания так называемых полусинтетических сред, в которых определенные индивидуальные химические вещества заменяются природными продуктами растительного или животного происхождения.

ТАБЛИЦА 1. СОСТАВ ИСКУССТВЕННОЙ ПИЩЕВОЙ СРЕДЫ ДЛЯ НАСЕКОМЫХ

TABLE 1. COMPOSITION OF ARTIFICIAL DIET FOR INSECTS

Группы веществ Groups of substances	Вещества Substances	Количество вещества (г) в 100 г среды Quantity in grams per 100 g nutrient medium
I. Азотистые вещества Nitrogenous substances	Казеин Casein	8,0
	Тирозин Tyrosine	0,015
	Валин Valine	0,015
	Аргинин Arginine	0,015
	Лейцин Leucine	0,015
	Лизин Lycine	0,015
	Аспарагиновая кислота Aspartic acid	0,004
	Аданин Adanine	0,01
	Метионин Methionine	0,01
	Серин Serine	0,01
	Фенилаланин Phenylalanine	0,01
	Цистин Cystine	0,15
	Глицин Glycine	0,15
II. Липиды Lipids	Растительное масло Vegetable oil	1,0
	Холестерин Cholesterine	0,1
III. Углеводы Carbons	Сахароза Saccharose	6,0
IV. Витамины Vitamins	Витамин B_1 B_1	0,0012
	Витамин B_2 B_2	0,0018
	Витамин B_3 B_3	0,0016
	Витамин B_{12} B_{12}	0,00006
	Пантотеновая кислота B_5 (pantothenic acid)	0,004

Таблица I (продолжение)

Группы веществ Groups of substances	Вещества Substances	Количество вещества (г) в 100 г среды Quantity in grams per 100 g nutrient medium
	Фолиевая кислота B_6 (folic acid)	0,001
	Никотиновая кислота Nicotinic acid	0,024
	Аскорбиновая кислота Ascorbic acid	0,03
	Витамин Е (токоферол) Vitamin E (tocopherol)	0,036
V. Прочие Others		
	Агар Agar	4,0
	Целлюлоза Cellulose	5,0
	Пивные дрожжи (автолизат) Brewer's yeast (autolysate)	8,0
	Смесь минеральных солей Mixture of mineral salts	2,0
	Вода Water	добавляется до 100 г added up to 100 g

Примером успешного создания простых полусинтетических сред является проведенная в СССР разработка сухой порошкообразной среды для Locusta migratoria [15]. Обычно в лабораторных условиях этот вид воспитывается на проростках пшеницы или других злаках, причем зеленый корм может быть частично заменен отрубями или другими органическими продуктами. Воспитание саранчи Locusta migratoria на синтетических средах проводилось как в Великобритании [16, 17], так и в СССР, и это позволило выяснить пищевые требования этого вида.

В результате ряда последовательных упрощений в конечном итоге была создана очень простая сухая среда, содержащая всего 7 компонентов: сухой тростник, сухое коровье молоко, отруби, сухие пивные дрожжи, сахарозу, аскорбиновую кислоту и холестерин [8]. На этой среде было воспитано несколько поколений саранчи, без добавления какого-либо зеленого корма, и получены нормальные особи с высокой плодовитостью.

Сухие порошкообразные среды обладают большими преимуществами так как не требуют заботы о борьбе с загниванием корма. Нет необходимости стерилизовать среду или добавлять в нее антисептики для предохранения от загнивания, что представляет собою одну из наиболее трудных задач при работе с искусственными средами. Однако, сухие среды могут использоваться лишь в ограниченном числе случаев – только для насекомых, способных активно пить воду, подобно саранче.

При работе с полусинтетическими средами большие перспективы сулит использование природных продуктов особенно богатых витаминами и аминокислотами.

Ряд авторов исследовал пищевую ценность пыльцы для некоторых насекомых [18 – 21].

Высокая пищевая ценность пыльцы была установлена в опытах с Anthonomus grandis [22]. Эти исследования показали, что пищевая ценность искусственных сред может значительно повышена путем добавления смеси цветочной пыльцы или зародыша семян пшеницы. Последний продукт также широко используется в исследованиях по искусственным питательным средам.

Особый интерес представляет маточное пчелиное молочко (royal jelly), которое чрезвычайно богато некоторыми жизненно важными для насекомых витаминами и аминокислотами. Недавними исследованиями Ремболда и его сотрудников [23 – 25] установлено исключительное богатство маточного молочка пантотеновой кислотой и фолиевой кислотой, т.е. витаминами, без которых развитие насекомых на искусственной пище не может успешно протекать. В то же время в природе крайне трудно найти другие продукты, которые были бы столь же богаты этими витаминами. К сожалению, до сих пор маточное молочко не использовалось при изготовлении искусственных сред.

При использовании в полусинтетических средах высушенных кормовых растений следует иметь в виду, что при сушке растений теряется значительная часть витаминов и стеринов, жизненно необходимых для развития насекомых, и поэтому они должны восполняться.

В работе Борисовой [15] было показано, что при высушивании тростника (Phragmites communis) для сухих сред, на которых воспитывалась Locusta migratoria происходят следующие изменения в содержании указанных веществ:

Поэтому для создания полноценной полусинтетической среды для Locusta migratoria в нее оказалось необходимым добавлять аскорбиновую кислоту и холестерин.

Хотя холестерин является продуктом животного происхождения и в растительных тканях не встречается, он является жизненнонеобходимым компонентом корма, так как успешно восполняет потребности насекомых в стеринах. Поэтому большинство рецептов синтетических и полусинтетических сред включают холестерин как обязательную составную часть.

Более детальные данные о влиянии отдельных пищевых компонентов на развитие насекомых-фитофагов приведены в указанных выше обзорах Эдельмана [5, 6].

Важнейшим вопросом при работе с искусственными средами является предохранение их от загнивания в течение длительного времени, необходимого для развития данного вида насекомого. Это достигается путем стерилизации посуды до помещения в нее пищевой среды и яиц или личинок насекомого (обычно с помощью автоклавирования), а также добавлением в среду специальных антисептиков, безвредных для насекомых. Большое количество различных антисептиков использовалось для предохранения сред от загнивания. Указания о химической природе таких антисептиков, применявшихся в различных странах, можно найти в обзоре Хауса [1]. В наших работах лучшим антисептиком оказалась смесь метилового эфира параоксибензойной кислоты с формальдегидом.

Трудоемкость процессов воспитания насекомых в массовых масштабах определяется, в первую очередь, затратами времени на стерилизацию посуды и садков и, в особенности, на смену корма при выращивании на-

ТАБЛИЦА 2. СОДЕРЖАНИЕ ВЕЩЕСТВ (мгр) НА 100 г СУХОГО ВЕСА

TABLE 2. CONTENT OF VARIOUS SUBSTANCES (mg) per 100 g OF CANE AS PERCENTAGE OF DRY WEIGHT

Вещества	Зеленый тростник	Высушенный тростник
Аскорбиновая кислота	117,975	35,893
Витамин Е	16,95	14,41
Стерины	0,232	0,114

Substance	Green cane	Dried cane
Ascorbic acid	117.975	35.893
Vitamin E	16.95	14.41
Sterols (solid alcohols)	0.232	0.114

секомых. Упрощение и автоматизация этих процессов является наиболее неотложной задачей в разработке методов массового разведения насекомых.

В наших исследованиях делается попытка разработать такой метод воспитания насекомых, который бы устранял необходимость смены корма в течение всего периода развития полной генерации данного вида. Это достигается помещением пищевой среды в стерилизованную стеклянную или пластмассовую посуду, которая после внесения в нее яиц или личинок насекомого, герметически закрывается на весь период личиночного и куколочного развития и контролизуется лишь после выхода имаго нового поколения. При этом учитывается, что количество помещенной в посуду пищевой среды должно не только обеспечить пищей личинку в течение всего периода ее развития, но и сохранить субстрат, необходимый для постройки колыбельки для куколки. Опыт показал, что для нормального развития одной особи такого размера, как Carpocapsa pomonella или Chloridea armigera достаточно герметически закрытое пространство объемом 0,030 – 0,040 литра.

Такая методика массового воспитания насекомых преследует еще и две другие цели: предотвратить гибель личинок вследствие каннибализма и развития эпизоотий. Конечно, массовое воспитание многих особей в общем помещении (садке или стакане) значительно упростило бы процесс воспитания и уменьшило бы затраты труда при этом. Но в этом случае предотвратить каннибализм и эпизоотии вследствие постоянного контакта особей почти невозможно. Поэтому в конечном итоге изолированное воспитание особей или выращивание их небольшими группами (2 – 5 особей) может иметь определенные преимущества. Можно наде-

яться, что дальнейшие исследования в этом направлении приведут к разработке таких методов индивидуального воспитания личинок, которые будут являться аналогией пчелиных сот, т.е. той системы воспитания, которая создана природой в результате многовекового естественного отбора.

Еще одним путем упрощения процесса массового разведения насекомых является воспитание их, в тех случаях, когда это возможно, на семенах растений, в частности на зерне злаков, т.е. на естественных продуктах, менее подверженных загниванию при длительном хранении. В этом отношении можно сослаться на опыт Энтомологического института Чехословацкой Академии Наук, где успешно разводятся в массе на семенах липы (Tilia) клопы Pyrrhocoris apterus и на семенах хлопчатника (Gossipium) – Disdercus intermedius, а также целый ряд других вредных насекомых.

Чехословацкими энтомологами подготовлено к печати руководство по методам лабораторного воспитания насекомых, которое содержит рецепты по культуре 70 видов насекомых [26]. Список видов, лабораторная культура которых осуществляется в Чехословакии, приведен в отчете Энтомологического института за 1964 – 1965 гг [27].

Аналогичным образом в результате многолетних исследований советских, иранских и французских энтомологов разработан метод воспитания черепашки (Eurygaster integriceps) на зерне пшеницы, который широко применяется для размножения паразитов яиц этого вида [28].

Другим важным вопросом массового разведения насекомых является разработка путей преодоления диапаузы у лабораторных популяций, что обеспечивает непрерывность разведения.

Что касается видов с факультативной диапаузой, то как показано исследованиями многих авторов, в особенности школой Данилевского [29, 30], возникновение диапаузы может быть предотвращено содержанием насекомых в условиях определенного фотопериода, т.е. при определенной длине светового дня с учетом влияния температурных условий.

Успешное разрешение этого вопроса можно проследить на примере плодожорки (Carpocapsa pomonella), для которой пути регулирования диапаузы детально изучены в результате многочисленных исследований фотопериодической реакции у этого вида американскими [31 – 33], советскими [29, 34, 35] и австрийскими [36, 37] исследователями.

Менее разработан вопрос устранения диапаузы для видов, имеющих в своем цикле развития облигатную диапаузу, т.е. для насекомых с моновольтинным циклом. При наличии облигатной диапаузы, обычно для ускорения прохождения этого состояния в реактивации диапаузирующей стадии, достаточно бывает выдержать ее в течение определенного времени в условиях пониженной температуры. Так, например, для случая облигатной эмбриональной диапаузы Locusta migratoria migrator достаточно бывает выдержать яйца этой саранчи в течение месяца при температуре ниже порога ее развития (16, 7°С), лучше при 4 – 10°С [38, 39]. Однако, известны немногие виды, как например Bombix mori, для реактивации диапаузирующих фаз у которых необходимы не низкие, а наоборот высокие температуры.

Возможен еще один путь устранения облигатной диапаузы не жизненного цикла насекомых. Этот путь практически намечен пока лишь единичными исследованиями, но теоретическая возможность его пока-

зана уже давно. Он основан на изменении генетической природы вида, путем скрещивания бездиапаузных и диапаузирующих рас одного и того же вида. Такие возможности очевидны лишь для ограниченного числа видов, имеющих достаточно обширные ареалы.

Теоретические возможности метода самоистребления насекомых, основанного на изменении их генетической природы, были показаны в работах ряда исследователей [40 – 42]. В последней работе [42] были указаны, в частности, возможности самоистребления, основанные на введении генов бездиапаузности в популяции видов, обычно развивающихся в природе с диапаузой; в результате зимующие фазы, теряющие способность диапаузировать, вымирают под воздействием низких температур вследствие их меньшей холодостойкости.

Конкретные возможности такого пути самоистребления насекомых пока-что выяснены лишь для двух видов Orthoptera: сверчка Teleogryllus commodus и саранчи Locusta migratoria.

Teleogryllus commodus широко распространен по всей Австралии. Специальными исследованиями установлено, что северная, квинслендская раса этого вида, обитающая в тропических условиях, развивается без диапаузы. Напротив, южная раса, обитающая в умеренных районах Австралии, имеет эмбриональную диапаузу. Опыты скрещивания этих рас показали, что бездиапаузность является доминантным признаком [43, 44]. На основании этого Хоган [45] предложил новый генетический метод борьбы с этим сверчком в умеренной зоне Австралии, основанный на подмешивании к природной популяции особей бездиапаузной, тропической расы. Другими словами он предложил способ введения новых генов в туземную популяцию, как метод снижения численности вредителя, в принципе сходны с методом выпуска стерильных самцов ("Sterile male technique"), только не требующий их облучения или обработки хемостерилянтами.

Другой пример подобного рода дает саранча Locusta migratoria, имеющая очень широкий ареал на всех континентах Старого Света. Эта саранча имеет целый ряд подвидов и рас, различающихся биологически. Обитающая в тропической Африке Locusta migratoria migratorioides, развивается без эмбриональной диапаузы и дает несколько генераций в году. Распространенная в СССР и в сопредельных странах Locusta migratoria migratoria, напротив, имеет облигатную диапаузу, благодаря чему имеет лишь одну генерацию в году. На территории Франции обитают три разновидности, различающихся характером эмбриональной паузы. Все это открывает большие возможности для скрещивания рас и для разработки путей самоистребления саранчи, аналогичных тем, которые предложил Хоган [45].

Проведенные нами опыты, а также исследования Ля Берре [46] во Франции показали, что все подвиды и расы L. migratoria легко скрещиваются между собой и эти результаты дают основание надеяться на успешную разработку этого нового метода борьбы с саранчой в ближайшем будущем.

В наших работах основное внимание уделяется разработке методов самоистребления и техники массового лабораторного разведения таких вредных насекомых, как Carpocapsa pomonella, Eurygaster integriceps и Locusta migratoria.

ЛИТЕРАТУРА
REFERENCES

[1] HOUSE, H.L., Artificial diets for insects: a compilation of references with abstracts. Inform. Bull. Res. Inst. Can. Dpt. Agric. (1967) 5.

[2] ШУМАКОВ, Е.М., ЭДЕЛЬМАН, Н.М., БОРИСОВА, А.Е., Опыт воспитания некоторых насекомых-фитофагов на искусственных средах. ДАН СССР 130 1 (1960) 237.
SHUMAKOV, E.M., EDELMAN, N.M., BORISOVA, A.E., Experience in feeding various plant-eating insects on artificial media, Dokl. Akad. Nauk SSSR 130 1 (1960) 237.

[3] UVAROV, B.P., Insect nutrition and metabolism. Trans. ent. Soc. Lond. 76 (1929) 255.

[4] HOUSE, H.L., The role of nutritional principles in biological control. Can. Ent. 98 (1966) 1121.

[5] ЭДЕЛЬМАН, Н.М., Питание насекомых на искусственных средах. Усп. совр. биологии 51 2 (1961) 204.
EDELMAN, N.M., The feeding of insects on artificial media, Usp. sovrem. biol. 51 2 (1961) 204.

[6] ЭДЕЛЬМАН, Н.М., Оценка влияния отдельных компонентов корма на развитие насекомых-фитофагов при воспитании их на искусственных средах. Зоол. Ж. 41 7 (1962) 1013.
EDELMAN, N.M., Assessing the effect of individual feed components on the development of plant-eating insects fed on artificial media, Zool. Zh. 41 7 (1962) 1013.

[7] SMITH, C.N., edit., Insect colonization and mass production, USA, 1966.

[8] ШУМАКОВ, Е.М., ЭДЕЛЬМАН, Н.М., БОРИСОВА, А.Е., Разведение фитофагов на искусственных средах. Защ. Раст. 6 (1967) 21.
SHUMAKOV, E.M., EDELMAN, N.M., BORISOVA, A.E., Rearing plant-eating insects on artificial media, Zashch. Rast. 6 (1967) 21.

[9] ФИЛИППОВИЧ, Ю.Б., К вопросу о связи белков корма с белками тела и шелка дубового шелкопряда. Уч. зап. Мос. Гос. Пед. Ин-та им. В.И.Ленина 98 (1957) 73.
FILIPPOVICH, Yu.B., The connection between feed proteins and body and silk proteins in the lappet moth, Uch. zap. Mosk. Gos. Pedagog. Inst-a im. V.I. Lenina 98 (1957) 73.

[10] ФИЛИППОВИЧ, Ю.Б., Усвоение, распад и новообразование аминокислот в организме шелкопряда. Биохимия 24 5 (1959) 904.
FILIPPOVICH, Yu.B., Uptake, decomposition and synthesis of amino acids in the silkworm organism, Biokhim. 24 5 (1959) 904.

[11] ФИЛИППОВИЧ, Ю.Б., Оценка питательного достоинства кормовых растений дубового шелкопряда при помощи аминограмм, ДАН СССР 131 4 (1960) 972.
FILIPPOVICH, Yu.B., Use of aminograms to assess the nutrient value of plants fed on by the lappet moth, Dokl. Akad. Nauk SSSR 131 4 (1960) 972.

[12] ФИЛИППОВИЧ, Ю.Б., Сравнительная оценка кормового достоинства для дубового шелкопряда листьев дуба, ивы, граба, орешника, березы и акации по аминокислотному составу их. В сб. "Биохимия шелкопряда", 1960, стр. 150.
FILIPPOVICH, Yu.B., Comparative assessment of the feed value derived by the lappet moth from leaves of oak, willow, hornbeam, nut-tree, birch and acacia, on the basis of their amino acid content, in "Biochemistry of the Silk-worm" (1960) 150.

[13] DETHIER, V.G., Host plant perception in phytophagous insects. Trans. IXth Int. Congr. Ent. 2 (1953) 81.

[14] LIPKE, H., FRAENKEL, G., Insect nutrition. Ann. Rev. Ent. 1 (1956) 17.

[15] БОРИСОВА, А.Е., Воспитание восточной и азиатской перелетной саранчи на полусинтетических средах. Зоол. Ж. 14 6 (1966) 858.
BORISOVA, A.E., Feeding the Eastern and Asiatic migratory locust on semi-synthetic media, Zool. Zh. 14 6 (1966) 858.

[16] DADD, R.H., Feeding behaviour and locusts. Adv. Insect. Physiol. 1 (1963) 47.

[17] CAVANAGH, G.G., The use of the Dadd synthetic diet as a food for adults Schistocerca gregaria (Forsk.) and the effect of some additions and modifications to it. J. Insect Physiol. 9 (1963) 759.

[18] HAYDAK, M.H., TANQUARY, M.C., Pollen and pollen substitutes in the nutrition of the honeybee. St. Paul, 1943.

[19] HAYDAK, M.H., Value of pollen substitutes for brood rearing of honeybee. J. econ. Ent. 38 4 (1945) 484.

[20] ИОЙРИШ, Н.П., "Пчелы – крылатые фармацевты", 1964.
IOIRISH, N.P., "Honey-bees – winged pharmacists" (1964).

[21] LEIUS, K., Effects of pollens on fecundity and longevity of adult Scambus buolianae (Htg.) (Hymenoptera: Ichneumonidae). Can. Ent. 95 2 (1963) 202.

[22] VANDERZANT, E.S., RICHARDSON, C.D., DAVICH, T.B., Feeding and oviposition by the boll weevil on artificial diets. J. econ. Ent. 52 (1959) 1138.
[23] REMBOLD, H., Über den Weiselzellenfuttersaft der Honigbiene. Verh. XI Int. Kongr. Entom. 3 (1960) 77.
[24] REMBOLD, H., Die Entstehung der Bienenkönigin. Umschau 61 16, 17 (1961) 488, 524.
[25] REMBOLD, H., Biologically active substances in royal jelly. Vitamins Hormones 23 (1965) 359.
[26] SKUHRAVÝ, V., edit., Metody chovu hmyzu, Praha (in press).
[27] Report on the activities of the entomological institute of the Czechoslovak Academy of Sciences for 1964-1965 (1966).
[28] REMAUDIERE, G., Plan for the installation and operation of a unit for the production of eggs of Eurygaster . Paris (1960) Sunn pest circ. 5.
[29] ДАНИЛЕВСКИЙ, А.С., Фотопериодизм и сезонное развитие насекомых. Л, Из. ЛГУ (1961).
DANILEVSKY, A.S., Photoperiodism and seasonal development of insects (1961).
[30] DANILEVSKII, A.S., Photoperiodism and seasonal development of insects (1965).
[31] DICKSON, R.S. et al. Continuous rearing of the Codling Moth. J. econ. Ent. 45 (1952) 66.
[32] PROVERBS, M.D., NEWTON, J.R., Effect of heat on the fertility of the Codling moth, Carpocapsa pomonella (L)(Lepidoptera: Olethreutidae). Can. Ent. 94 3 (1962) 225.
[33] PUTMAN, W.L., The codling moth, Carpocapsa pomonella (L.)(Lepidoptera: Tortricidae): a review with special reference to Ontario. Proc. ent. Soc. Ontario 93 (1962) 22.
[34] ШЕЛЬДЕШОВА, Г.Г., Значение длины дня в регуляции числа поколений и диапаузы яблонной плодожорки Laspeyresia pomonella L. ДАН СССР 147 (1962) 480.
SHELDESHOVA, G.G., The part played by length of daylight in regulating the number of generations and the diapause of the lesser apple worm Laspeyresia pomonella L., Dokl. Akad. Nauk SSSR 147 (1962) 480.
[35] ШЕЛЬДЕШОВА, Г.Г., Географическая изменчивость фотопериодической реакции и сезонного развития яблонной плодожорки Laspeyresia pomonella L. (Lepidoptera, Tortricidae) Тр. Зоол. ин-та, Л 36 (1965) 5.
SHELDESHOVA, G.G ., Geographic variability of the photoperiodic reaction and seasonal development of the lesser apple worm Laspeyresia pomonella L. (Lepidoptera, Tortricidae), Tr. zool. Inst-a 36 (1965) 5.
[36] RUSS, K., Der Einfluss der Photoperiodizität auf die Biologie des Apfelwicklers (Carpocapsa pomonella L.). Pfl. schutz-Ber., Sonderheft (1966) 27.
[37] RUSS, K., Neue Erkenntnisse über die Biologie und die Bekämpfung des Apfelwicklers (Carpocapsa pomonella L.). Der Erwerbsobstbau 9 (1967) 21.
[38] ЯХИМОВИЧ, Л.А., Смена требований к условиям среды в процессе эмбрионального развития азиатской саранчи. ДАН СССР 73 5 (1950) 1105.
YAKHIMOVICH, L.A., Adaptation to environmental conditions in the embryonic development of the Asiatic locust, Dokl. Akad. Nauk SSSR 73 5 (1950) 1105.
[39] ЯХИМОВИЧ, Л.А., Развитие яиц и созревание азиатской саранчи в связи с условиями существования. Диссертация, Ленинград, ВИЗАР, 1952.
YAKHIMOVICH, L.A., Egg development and maturation of the Asiatic locust as a function of environmental conditions, Doctoral thesis, Leningrad, VIZAR (1952).
[40] СЕРЕБРОВСКИЙ, А.С., О новом возможном методе борьбы с вредными насекомыми. Зоол. Ж. 19 4 (1940) 618.
SEREBROVSKY, A.S., A possible new method of combatting insect pests, Zool. Zh. 19 4 (1940) 618.
[41] KNIPLING, E.F., Use of insects for their own destruction. J. econ. Ent. 53 3 (1960) 415.
[42] LA CHANCE, L.E., KNIPLING, E.F. Control of insect populations through genetic manipulations. Ann. ent. Soc. 55 5 (1962) 515.
[43] BIGELOW, R.S., Factors affecting developmental rates and diapause in field crickets. Evolution 16 (1962) 396.
[44] HOGAN, T.W., Some diapause characteristics and interfertility of three geographic populations of Teleogryllus commodus (Walk.). Austral. J. Zool. 13 3 (1965) 455.
[45] HOGAN, T.W., Physiological differences between races of Teleogryllus commodus (Walker) (Orthoptera: Gryllidae) related to a proposed genetic approach to control. Austral. J. Zool. 14 2 (1966) 245.
[46] LE BERRE, J.R., Caractères biologiques des Locusta de la Faune de France et étude d'un exemple de diapause embryonnaire. Annal. épiph. 10 2 (1959) 101.

DISCUSSION

Rachel GALUN: You say that phagostimulants are less important than appropriate selection and proportions of the basic ingredients in the nutrient medium. House, however, emphasized in the course of an Agency panel in 1966* the importance of phagostimulants for monophagous insects. I think the reason for this discrepancy is that the insects you mention have nutritional components as stimulants – for example, ascorbic acid for locusts, cholesterol for the silkworm, and glucose and amino acids for the corn borer.

I should also like to point out that further economy of mass rearing can be obtained by using the medium on which the insects were reared to feed pigs or geese, as much of its nutritive value is retained.

E. M. SHUMAKOV: Thank you for your comments. The suggestion of re-using the nutrient medium is an interesting one. As the paper points out, the artificial medium is used not only as food, but also as an environment for pupation, etc. Hence a considerable part of it is, in fact, not really used, and its nutritive qualities could indeed be put to further use.

* Isotopes and Radiation in Parasitology (Panel Proceedings), IAEA, Vienna (1968) in the press.

THEORETICAL AND PRACTICAL STUDIES ON A POSSIBLE GENETIC METHOD FOR TSETSE FLY CONTROL

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Abstract

THEORETICAL AND PRACTICAL STUDIES ON A POSSIBLE GENETIC METHOD FOR TSETSE FLY CONTROL. Chromosome translocations may be useful in pest control because they are a common type of mutation in a variety of organisms and, frequently, the heterozygote is semi-sterile and the homozygote fully fertile. It might be possible to induce such a translocation in a pest species, to breed from a selected ancestral pair of translocation homozygotes a large number of the homozygotes and to release these into a wild population. This would cause the production of heterozygotes in the wild population and hence would reduce the fertility of the population. This reduction would persist for a number of generations. Calculations, based on simplified assumptions, showed that this method of fertility reduction might be more economical than the use of sterilized males.

In the present paper a theoretical comparison is made of the translocation and sterilized-male methods for the control of tsetse flies (Glossina sp.). A computer model has been set up which simulates, as far as possible, the known facts about birth, mating and death in a wild tsetse population. The predicted effects of releases of sterilized males and of translocation homozygotes are described and the modifications which would be caused by density-dependent mortality, migration and reduced viability of the translocation genotypes and sterilized males are indicated. It is concluded that to eradicate a well isolated wild population the numbers of translocation homozygotes required might well be considerably less than the number of sterilized males required for the same task. However, immigration into the population would greatly reduce the efficiency of the translocation method.

The progress so far in attempting to produce a suitable translocation in Glossina austeni is described. Males have been treated with 5-7 krad of gamma radiation and a number of semi-sterile individuals have been selected from among their progeny. The semi-sterility is inherited and, by analogy with the results in other organisms, is presumed to be due to translocation heterozygosity. The methods being used to attempt to derive translocation homozygotes from the semi-sterile lines are described.

1. INTRODUCTION

Chromosome translocations can readily be induced by radiation in a wide variety of organisms. Translocation heterozygotes are frequently found to be semi-sterile, whereas translocation homozygotes, provided that they are viable, are generally found to be fully fertile. The chromosomal basis of these properties is well known [1]. If a suitable translocation were induced in a pest species and if a male and a female homozygous for the translocation were obtained, a large number of the homozygotes could, in principle, be derived from this pair. Release of homozygotes of both sexes into a wild population would lead to matings

between translocation homozygotes (T/T) and wild type homozygotes (+/+) which would produce translocation heterozygotes (T/+) and hence a reduction in the fertility of the wild population in the next generation. As long as the translocation and normal chromosomes persisted in the population, heterozygotes would continue to be produced in subsequent generations and the fertility of the population would be depressed. Natural selection acts in a peculiar way in a population containing translocation and normal chromosomes [2, 3]. If the T/+ and T/T individuals are assumed to have normal viability and mating competitiveness, whichever of the two chromosome types is in the majority when the release programme is finished will gradually increase in frequency until it reaches fixation. There is a theoretical point at exact equality of the frequencies of the two chromosome types when they would both persist indefinitely in the population, but this is an unstable equilibrium. At this frequency the reduction in population fertility is maximal.

Calculations were presented in a previous paper [3] of the effects on population size of releasing various numbers of translocation homozygotes into a given population with the above simple assumptions about viability and mating competitiveness as well as the following ones: (a) the generations in the treated population do not overlap, (b) there is a 1 : 1 sex ratio, (c) no density-dependent factors operate, (d) the treated population is isolated from migration, and (e) all adult females are inseminated. The results were compared with the calculated effects of releasing sterile males into the population and it was concluded that on these assumptions considerably fewer insects would have to be reared to obtain a given effect with the translocation method than with the sterilized male method. This advantage arises partly from the long continued effect of the translocations and partly from the fact that the release of female translocation homozygotes would contribute usefully to population reduction, whereas the release of sterilized females would not.

We were interested in the possible application of the translocation method to tsetse fly control, and it seemed important to repeat these theoretical comparisons with more realistic assumptions about tsetse populations. A theoretical model of a tsetse population was previously used to predict the effect of the sterilized male method on tsetse flies [4]. In the present study we also constructed a population model and the release of either sterilized males or translocation homozygotes could be simulated with a computer. The effects of removing some of the other assumptions listed above have also been tested.

In the last section of the paper, attempts to produce a suitable translocation in the tsetse species Glossina austeni are described.

2. THE MODEL

We assumed that the size of the population to be treated was very large so that a deterministic model could be used.

As far as possible the model was based on data about tsetse flies given in the standard text-book on the genus [5], but, where the facts were not definitely known, guesses have had to be made.

A diagram of the population model before any releases are made is shown in Fig. 1. Without treatment we assumed that the population size

stayed constant so the diagram represents both the history of a cohort born at one time or the composition of the whole population at any time. In Fig.1 and throughout the rest of the paper, numbers of individuals are expressed in units defined relative to the total initial female population which consists of 100 units. The continuous process of birth and death in the population was approximated by a discrete model with a

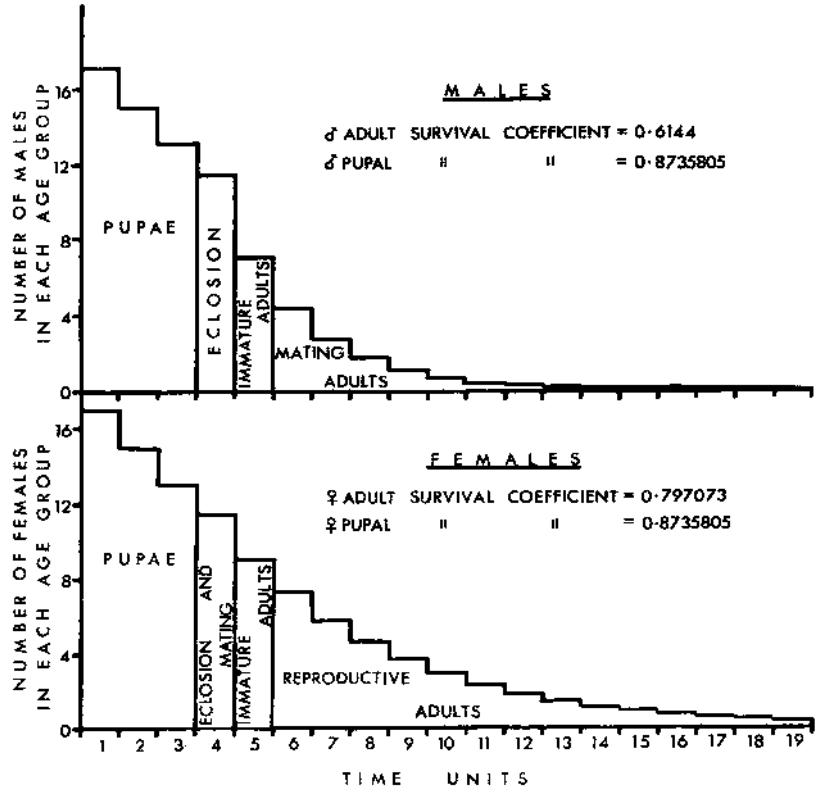

FIG. 1. Model of a wild tsetse population.

time interval of 10 days, which we call the time unit. This period was chosen because it is the average time between successive ovulations in an adult female, and in the untreated population surviving females of reproductive age are assumed to produce one pupa per time unit and the sex ratio of these is assumed to be 1 : 1. The pupal survival coefficient, i.e. the proportion of pupae surviving at age n time units which survive to n+1, was assumed constant and was chosen to give an overall survival of $\frac{3}{4}$ from the time of deposition up to the time of eclosion. Up to age 19 time units the adult survival coefficient, i.e. the proportion of adults of age n time units which survive to age n+1, was assumed to be a constant for each sex. The few survivors at age 19 time units were assumed to die of old age. The male adult survival coefficient was chosen to conform with data on the later age groups of wild male G. morsitans [6].

Because the size of the untreated population was required to stay constant it was necessary to choose the precise value for the female adult survival coefficient which satisfies this condition. The adult sex ratio generated by the model is 1 male : 1.85 females, which conforms with the general opinion that there is a considerable excess of females in a tsetse population. Females were assumed to be inseminated, at the age indicated in Fig. 1, by a random sample of the surviving mature males and the sperm received was assumed to be used for the rest of their reproductive life.

The sterilized males and translocation homozygotes were assumed to be released when aged 4 time units, and the releases were assumed to be distributed evenly throughout the treated population.

The translocation karyotypes, sterilized males and wild types were assumed to mate with each other at random and the sterilized sperm and the various types of gamete produced by the translocation karyotypes were assumed to have normal fertilizing capacity. The latter assumption conformed with observations on radiation-sterilized tsetse sperm [7] and translocations in Drosophila [8]. The assumed outcomes of the matings between the translocation and normal karyotypes are shown in Table I.

We have assumed that the death of a zygote in a female, as a result of the action of a translocation or sterilized sperm, does not affect the time of her next ovulation or prolong her life. The former effect has been detected [7, 9] but was relatively small and the latter effect was not detected in experiments on young flies [7].

The influence that three factors would have on the effectiveness of the control methods was investigated on the following simple assumptions about their modes of operation:

TABLE I. THE ASSUMED OUTCOME OF MATINGS BETWEEN THE TRANSLOCATION KARYOTYPES AND THE WILD TYPE

Mating	Progeny			
	+/+	T/+	T/T	Inviable
+/+ × +/+	1	0	0	0
T/+ × +/+	½	½	0	½
T/T × +/+	0	1	0	0
T/+ × T/+	1/16	3/16	1/16	11/16
T/+ × T/T	0	¼	¼	½
T/T × T/T	0	0	1	0

(a) Viability. The adult survival coefficients for sterilized males or for T/+ or T/T individuals could be multiplied by a chosen factor which we call the relative viability coefficient.

(b) Density dependent factors. The male and female adult survival coefficients at time t were multiplied by:

$$1+d(1 - N_t/N_0)$$

where N_t is the population size at time t, N_0 is the size of the untreated population, and d is called the 'density dependence coefficient' and may be specified at will.

When $d>0$ the population tends to return to N_0 after any temporary disturbance.

(c) Migration.[1] The tendency to migrate was assumed to be independent of adult age. We defined the 'emigrant proportion' as the proportion of adults of age n time units which emigrated from the population before age n+1. This quantity was separately specifiable for each sex. In the untreated population the immigration rate was made to balance the chosen emigration rate. As the population was reduced, the absolute number of emigrants fell, but the number of immigrants, since they were assumed to come from surrounding untreated populations, remained constant. Migration therefore acted as a population stabilizing influence. Emigrants were assumed to be a random sample of the adult flies in the treated population, but immigrants were assumed to be fertile wild types only and, because females are known to be inseminated early in adult life, female immigrants were all assumed to be inseminated with fertile wild type sperm.

3. RESULTS OF THE COMPUTING

As a check on the correct performance of the computer model, releases of equivalent numbers of sterilized males to those used by Simpson [4] were tried and similar results were obtained.

The release of sterilized males would cause a reduction in fertility of the population until all females inseminated with sterilized sperm were dead, but with the translocation method the fertility reduction would continue into later generations until the population had become homozygous either for the normal chromosomes or the translocation chromosomes. In some cases it was found that this process would take several years after the last release of translocation homozygotes.

The simulated release of a given total of sterilized males or translocation homozygotes was found to have a greater effect the more frequent the releases and the longer the total time over which releases were made. It was decided in all the succeeding computer trials to use 10 equal releases made at intervals of four time units, which represented a release programme extending over about one year.

Releases of approximately equal numbers of T/T males and females were found to give almost the same long-term result as release of the same total number of males only. It has been shown [10] that the output

[1] 'Migration' is used in the sense of diffusion and not orderly movement.

of progeny for release from a captive colony of tsetse flies could consist of young adult males and females in the ratio 0.54 : 0.46 without depleting the breeding stock, and all releases of translocation homozygotes were assumed to be made in this ratio.

The effort involved in collecting enough tsetse pupae from the wild for use in a sterilized male release programme is very great [11], and it seems that with modern rearing methods [10, 12] it would be more economical to rear the necessary males in a captive colony. When comparing the translocation and sterilized male methods, comparison should be made between the numbers of males of each type that are needed for release, since this indicates the respective sizes of the captive colonies that would have to be maintained and therefore gives a rough indication of the relative cost of the two methods.

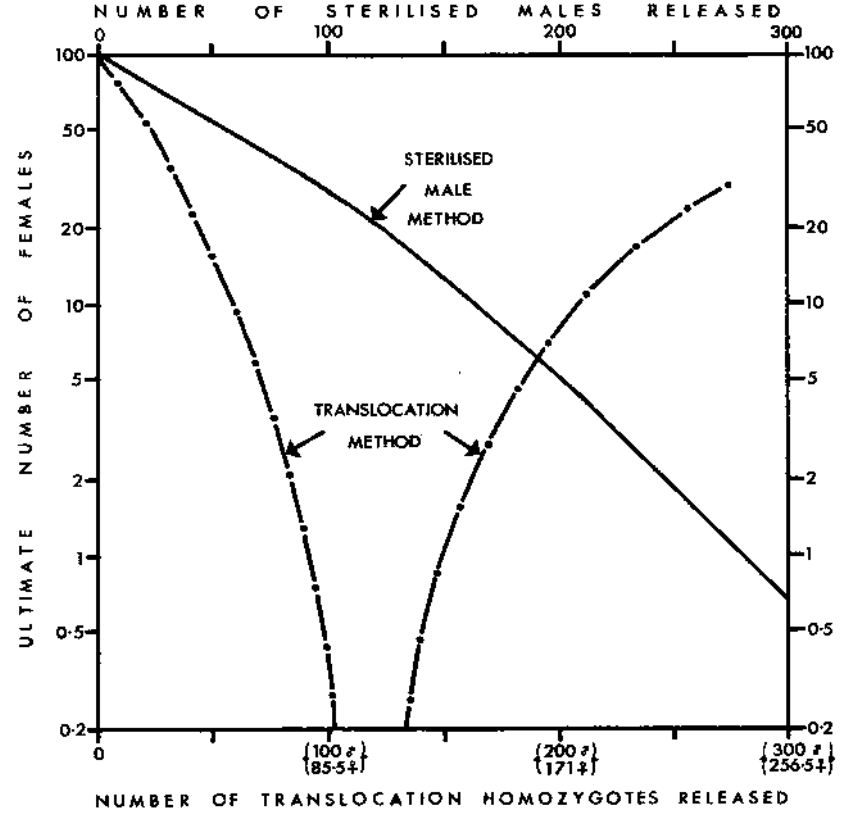

FIG. 2. Total numbers of units of sterilized males or translocation homozygotes released plotted against the ultimate size of the female population.

Releases of various numbers of sterilized males and translocation homozygotes were simulated, normal viabilities and no density dependence or migration being assumed; the total numbers released are plotted in Fig. 2 against the female population size when it stabilized, which we

call the ultimate number of females. The ultimate number of females declined steadily with increase in the number of sterilized males released. With the translocation method there is a very sharp optimum number to be released because of the peculiar response of translocations to natural selection which has already been described. With the assumptions used, unless the optimum is exceeded the translocation method shows a great advantage in efficiency over the sterilization method. The superiority of the translocation method is seen to depend critically on releasing the correct number of homozygotes. It would be very laborious to estimate the size of the population to be treated with sufficient accuracy so that the numbers of flies to be released could be specified exactly in advance. However, if suitable techniques were developed it might be possible to estimate cytologically the frequency of the translocation chromosome in the wild population as the release programme proceeded and to adjust the numbers to be released so as to approach the optimum frequency of 0.5.

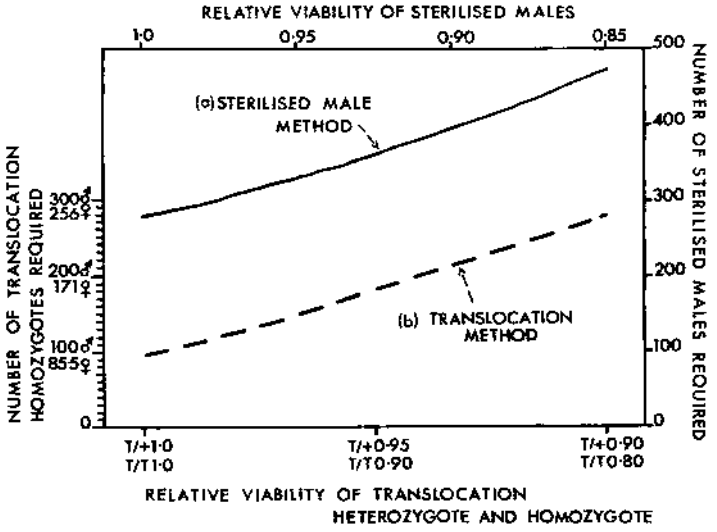

FIG. 3. The numbers of (a) sterilized males or (b) translocation homozygotes required to be released to achieve the target population reduction plotted against values of the relative viability coefficient for sterilized males or T/+ and T/T individuals.

It is probable that the direct effects of the release of sterilized males or translocations are only required to reduce the population to some very low density and that other factors, such as the inability of the flies to find mates or the ecological effects of human occupation of the land will then intervene to eradicate the surviving flies [5]. Therefore, to study the effects of viability differences, density-dependent factors and migration, we assumed that the target was to reduce the population of females, as a direct result of the releases, to one unit. For each set of parameters the number of flies required to reach this target was estimated. For simplicity the effects of each of the three factors, viability, density dependence and migration, were investigated in the absence of the other two.

Figure 3 shows the numbers of sterilized males or translocation homozygotes that would be required to be released at various values of the relative viability coefficient (as defined above) of sterilized males or the translocation karyotypes. The effect of any decrease in viability can, in principle, be overcome by releasing a sufficiently large number of flies.

There is, in fact, evidence [13] that it is possible to sterilize tsetse flies with Tepa without any loss of viability in captivity, but data on the viability of such flies in the wild have not yet been published. In Drosophila, translocation homozygotes frequently show low viability [14]; however, different translocations vary in this respect. Since only a single translocation would be required to establish a captive colony of homozygotes, it seems reasonably likely that one with suitable properties could be selected in Glossina.

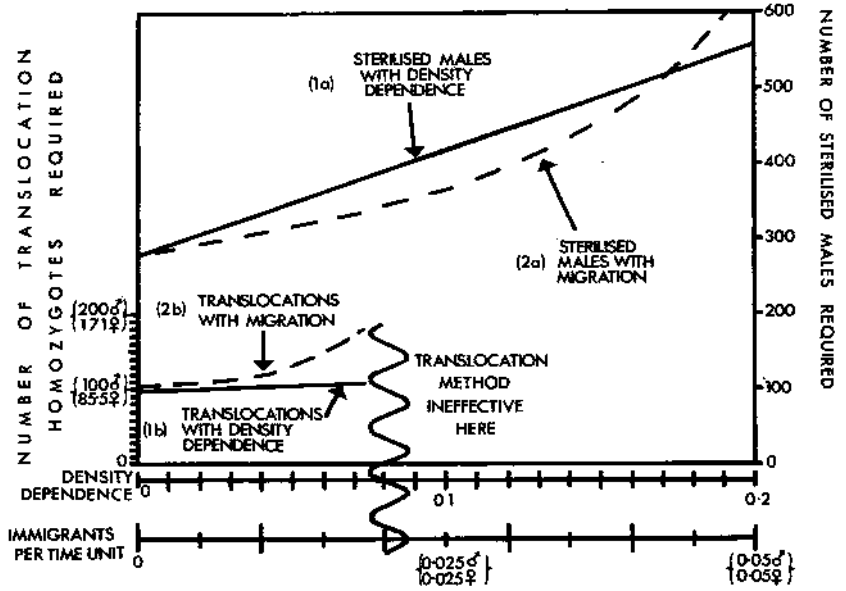

FIG. 4. The numbers of (a) sterilized males or (b) translocation homozygotes required to be released to achieve the target population reduction plotted against values of (1) the density dependence coefficient and (2) the immigration rate.

The numbers of flies required to reach the target population reduction in the presence of various levels of the density dependence coefficient are plotted in Fig. 4. Any degree of density dependence could, in principle, be overcome by the release of sufficient sterilized males. However, if the coefficient exceeds about 0.075 the required population reduction cannot be achieved by the translocation method, because at higher levels of density dependence the increase in viability which occurs as the population density falls overcomes the maximum reduction in fertility that can be obtained by the action of a translocation. Unfortunately, little quantitative evidence exists on which to decide how strongly density-dependent factors operate in wild tsetse populations [15]. How-

ever, Hocking [16] reduced an isolated tsetse population to 1% of its initial density with a non-residual insecticide. Human occupation of the area did not occur. The tsetse population density remained at about 1% of that of a control (unsprayed) area over a six-year period. A density dependence coefficient of 0.075 would have increased the population from 1 unit to 99.74 units over this period; it appears that, at least in some tsetse populations, density-dependent factors would not be strong enough to overcome the operation of the translocation method.

Figure 4 also shows the releases required to achieve the target population reduction at various levels of migration. The same number of male and female migrants per time unit were assumed, implying a considerably higher rate of male migration per head of the adult population. This ratio was chosen to represent the apparently higher activity of males in wild tsetse populations [5]. As already explained, migration has a population stabilizing tendency so that, as with density-dependent factors, there is a critical level of migration above which it is impossible to achieve the desired target with the translocation method. There is no such critical level with sterilized males, but the numbers required rise very sharply with the migration rate. Jackson [17] made a quantitative study of migration for wild male G. morsitans and concluded that about 1.5 units of males per time unit (using the terminology of this paper) were permanent emigrants from a 16 square mile block marked out in a much larger area of tsetse occupied land. It is clear from Fig. 4 that it would be useless to treat with translocations such a small area within a large tsetse fly 'belt', since where the immigration rate exceeds about 0.018 units of each sex per time unit, the translocation method becomes ineffective. Tsetse are, however, distributed in a discontinuous way because of variations in the suitability of the environment.

The rates of migration across unsuitable environments must be very much less than those found by Jackson. Artificial clearance of barriers can certainly reduce migration to very low rates, since the population referred to already [16], which was reduced to 1% of its initial density, would have increased seven fold over the six year period if an immigration rate of 0.018 units of both sexes/time unit had occurred. No increase was, however, detected. This population only extended over a small area and we remain uncertain whether tsetse populations of veterinary or medical importance exist which are sufficiently isolated, or which could be made so economically, to allow control by the translocation method to be successful.

4. ATTEMPT TO PRODUCE A SUITABLE TRANSLOCATION IN Glossina austeni

There are no genetic markers available in Glossina, and so the method used to attempt to produce and select translocations was essentially the same as that used for the same purpose in mice [18].

Adult male G. austeni were irradiated with 5-7 krad of ^{60}Co gamma radiation and mated immediately to unirradiated females. Thus paternal chromosomes of the F_1 were irradiated while in mature spermatozoa.

Dominant lethals were induced in about 80% of the irradiated sperm but the remainder gave rise to F_1 individuals. The fertility of the F_1 males was tested by mating each to normal females and recording their pupal production for a standard time; cases of reduced fertility were then selected. Glossina has the advantage for this work that females ovulate one egg at regular intervals, and when the females are well fed and have been mated to a normal male, the probability that the egg will give rise to a pupa is high [7, 12]. Cases where genetic abnormality causes deaths of embryos are therefore fairly easily distinguishable. About 20 F_1 males with reduced fertility have been obtained, and although in most cases the fertility is about one half of normal, in a few cases it is very much less than this. About one half of the sons of these semi-sterile males inherited the semi-sterility and by analogy with the results in other organisms it seems likely that the semi-sterility is due to translocation heterozygosity. Cytological confirmation of this has not yet been attempted.

The mates of one of the semi-sterile males and those of several of its sons produced a large proportion of aborted larvae. It is suggested that the aneuploid chromosome sets which this translocation produced was not always lethal until late in embryonic development.

It was unexpectedly found that a large proportion of the daughters of the semi-sterile males showed total sterility. A few females with partial sterility were, however, obtained, and preliminary results suggest that they transmit this character to their offspring in the manner expected of a translocation heterozygote. If it is assumed that this is confirmed, the procedure to attempt to produce translocation homozygotes will be to inbreed the offspring of a semi-sterile female and to test the fertility of each mating. One son of each fully fertile mating will be test-mated to wild type and if this produces all semi-sterile offspring, this would indicate that a line homozygous for a translocation had been produced, and the rearing of a colony of translocation homozygotes could then begin.

REFERENCES

[1] BURNHAM, C.R., Discussions in Cytogenetics, Burgess, Minneapolis (1962). Ch. 4.

[2] WRIGHT, S., Am. Nat. 75 (1941) 513.

[3] CURTIS, C.F., Bull. Ent. Res. (1967) in press.

[4] SIMPSON, H.R., Biometrics 14 (1958) 159.

[5] BUXTON, P.A., The Natural History of Tsetse Flies, Lewis, London (1955).

[6] JACKSON, C.H.N., Bull ent. Res. 39 (1948) 441.

[7] CURTIS, C.F., paper in preparation.

[8] MULLER, H.J. and SETTLES, F. Zeit. ind. Abst. Verherb. 43 (1927) 285.

[9] SAUNDERS, D.S., Trans. R. ent. Soc. Lond. 112 9 (1960) 221.

[10] JORDAN, A.M. and CURTIS, C.F., Bull. ent. Res. (1967) in press.

[11] DEAN, G.J.W., personal communication.

[12] JORDAN, A.M., NASH, T.A.M. and BOYLE, J.A., Ann. trop. Med. Parasit. 61 (1967).
[13] DAME, D.A., and FORD, H.R., Bull. ent. Res. 56 (1966) 649.
[14] IVES, P.T. and FINK, G.R., Genetics 47 (1962) 963.
[15] GLASGOW, J.P., The Distribution and Abundance of Tsetse, Pergamon, Oxford (1963).
[16] HOCKING, K.S., XI Int. Kong. Ent. Wien 1960 Bd. II (1962) 409.
[17] JACKSON, C.H.N., Bull. ent. Res. 32 (1941) 53.
[18] CARTER, T.C., LYON, M.F. and PHILIPS, R.J.S., J. Genet. 53 (1955) 154.

APPENDIX

COMPUTING METHOD

It is sufficient to specify the changes taking place in the population when the time advances by one time unit.

Translocation method

Let:-

Q_{sknt} = the number of individuals of sex s (s = 1 for males, s = 2 for females) of karyotype k (k = 1, 2, 3 for +/+, T/+, T/T respectively) of age n time units at time t time units. No T/T flies are released until $t \geq 1$.

p = the pupal survival coefficient; $p = 0.8735805 = (\frac{2}{3})^{\frac{1}{3}}$.

a_s = the adult survival coefficient for sex s; $a_1 = 0.6144$, $a_2 = 0.797073$.

X_{st} = the number of T/T flies of sex s released at time t.

f_{ijk} = the frequency of progeny of karyotype k from a mating of individuals of karyotype i and j (see TABLE 1).

v_k = the relative viability coefficient for karyotype k.

d = the density dependence coefficient.

m_s = the emigrant proportion for adults of sex s.

M_s = the number of immigrants of sex s.

N_t = the population size at time t and is given by

$$N_t = \sum_{s=1}^{2} \left[\sum_{k=1}^{3} \sum_{n=1}^{19} Q_{sknt} + M_s \right]$$

When $t>0$ the adult mortality can be expressed by:

$$(1)\quad Q_{sk,n+1,t+1} = a_s \; v_k \left[1+d\,(1-N_t/N_0)\right] (1-m_s)\; Q_{sknt}, \qquad 4 \leqslant n \leqslant 18$$

$$(2)\quad Q_{sknt} = 0, \qquad n > 19$$

and the pupal mortality can be expressed by:

$$(3)\quad Q_{sk,n+1,t+1} = p\; Q_{sknt}, \qquad 1 \leqslant n \leqslant 3$$

except when T/T individuals are released when:

$$Q_{s34,t+1} = p\,Q_{s33t} + X_{s,t+1}$$

To compute the numbers of new births we need to define u_{jknt} = the expected proportion of the progeny of a female of karyotype j of age n ($4 \leqslant n \leqslant 19$) at time t which have karyotype k. This quantity depends on the frequencies of the types of male in the population when the female mates and is given by:

$$u_{jk4t} = \frac{\left[\sum_{i=1}^{3} f_{ijk} \sum_{n=5}^{19} Q_{lint} + f_{1jk}\; M_1\right]}{\left[\sum_{n=5}^{19} \sum_{i=1}^{3} Q_{lint} + M_1\right]}$$

$$u_{jk,n+1,t+1} = u_{jknt}, \qquad 5 \leqslant n \leqslant 19$$

The new births of pupae are therefore:

$$Q_{sklt} = \tfrac{1}{2} \sum_{j=1}^{3} \sum_{n=6}^{19} Q_{2jnt}\; u_{jknt}, \qquad k \neq 1$$

$$Q_{s11t} = \tfrac{1}{2} \left[\sum_{j=1}^{3} \sum_{n=6}^{19} Q_{2jnt}\; u_{j1nt} + M_2\right]$$

The above formulae were used repeatedly to study long term changes in the population and an electronic computer was needed to handle the calculations.

Sterilised male method

The above formulae can easily be modified to apply to the sterile male method:

k is re-defined so that k = 1 for normal individuals and k = 2 for sterilised males.

Y_t is defined as the number of sterilised males released at time t ($t>0$).

Equations (1) (2) and (3) hold without change except that k can only take the value 1 in (3). Release of sterilised males gives:

$$Q_{124t} = Y_t$$

If we define u_{nt} $(4 \leqslant n \leqslant 19)$ as the proportion of females of age n which have mated with a fertile male, then:

$$u_{4t} = \frac{\left[\sum_{n=5}^{19} Q_{11nt} + M_1\right]}{\left[\sum_{i=1}^{2} \sum_{n=5}^{19} Q_{1int} + M_1\right]}$$

$$u_{n+1,t+1} = u_{nt}, \qquad 5 \leqslant n \leqslant 19$$

and:

$$Q_{s11t} = \tfrac{1}{2}\left[\sum_{n=6}^{19} Q_{21nt}\ u_{nt} + M_2\right]$$

DISCUSSION

R.C. VON BORSTEL: The system you suggest could be made formally identical to the irradiation-of-male method, by releasing males (but not females) with multiple translocations. Next time, males with different sets of multiple translocations could be released; in each case most of the daughters and sons would be heterozygous, and thus sterile. After one translocation was made homozygous, this stock could be used again to obtain more translocations, and gradually the desired lines could be achieved. By using males from different stages of the developing multiple translocation lines for the releases, the advantages of both the lethal male and the translocation male systems could be obtained.

C.F. CURTIS: The release of flies from several stocks carrying different multiple translocations would certainly be more efficient than the system I have described, provided that viable multiple homozygotes could be obtained. I agree that the best way to produce multiple translocation homozygotes would be to try to accumulate the translocations in successive steps, but because of the long generation times in tsetse this process would, of course, take a long time. I think it possible that the stocks with fertility well below 50% that I mentioned may perhaps be cases in which two or more translocations have arisen at once. Cytological data and evidence as to whether these 'high sterile' lines segregate 50% sterile offspring are not yet available.

V. LABEYRIE: Have you tested the validity of your working hypothesis that mating is random? Are the irradiated males not at a disadvantage compared with normal males, and does not this disadvantage depend on

the ratio of various types present? If so, surely the minority males have an advantage, as has been shown for D. melanogaster by Petit, and confirmed in Dobzhansky's laboratory.

C.F. CURTIS: We have not tested, with the computer, the effects of non-random mating. I would guess that an overall reduction of the mating competitiveness of the translocation individuals would give much the same result as a reduction in viability of these individuals and, as Fig. 3 of the paper shows, this can be overcome by the release of more homozygotes. The occurrence of assortative mating might be much more harmful, since if individuals tended to select flies of their own type as mates, the proportion of heterozygotes, and hence the sterilizing effect, would be reduced. There would be strong natural selection for the evolution of such assortative mating in a population being attacked by means of translocations, but recognition of the different chromosome types by the insects might be difficult, because they are not expected to differ morphologically.

D.T. NORTH: Have you considered examining your induced translocations to determine whether any of them show a bias towards adjacent segregation as opposed to alternate segregation? Multiple translocations are not only difficult to create, particularly when genetic markers are not available, but are often difficult to maintain in a homozygous form. It would appear to me that the search for segregation directed towards adjacent segregation of the translocation heterozygote at meiosis would not involve much additional work, and might have more practical value than the manipulation and induction of multiple translocation stocks.

C.F. CURTIS: I agree that single translocations with less than 50% fertility in the heterozygote would be extremely useful, but we thought, when setting up our computer model, that it would be over-optimistic to assume the existence of such a mutation. If such a translocation could be obtained, it would, of course, help to overcome the effects of any density-dependent factors or immigration that might occur in the wild population it is desired to eradicate.

D.A.T. BALDRY: Although Hocking (Ref. [16] of your paper) has reported that a tsetse population can be reduced to a low level and can remain at that reduced level for 6 years, we know only too well, after many unsuccessful attempts to control tsetse by insecticide methods, that residual populations rapidly increase in density. From my experience I think it unlikely that human settlement and development of a tsetse fly belt would eliminate the remaining 1 or 2% of a tsetse population that had been reduced by active control methods. Such residual tsetse populations are extremely difficult to eliminate by any method.

I also have a question. Could you please describe your technique for estimating the number of translocated tsetse flies, and the number of experimental animals required to maintain them, that would be needed to eliminate a tsetse population of a known density occupying a fly-belt of known size?

C.F. CURTIS: If density-dependent factors operate in one tsetse population one would expect them to operate in all. If Hocking found one population where they did not operate, I would be inclined to think, in the absence of absolute certainty that the populations you refer to are completely isolated, that the recoveries after insecticide attack may have been due to immigration. It is rather difficult to believe that tsetes

compete for food, and the evidence on predation suggests that they do not form a substantial proportion of the food of their predators (Ref.[15] of the paper). I agree, however, that on present data it is not possible to exclude the possibility that density-dependent factors do operate in tsetse at a low level.

I wonder whether the low-density residual populations, provided they do not increase again, are of medical or veterinary importance in trypanosome transmission. It might be found useful after a population has been reduced to a low level by the translocation method to eradicate the survivors by release of sterile males. Of course the numbers of sterile males required would then be relatively low; with a low population it would probably be difficult to assess the correct number of translocations to be released, but in the case of sterile males it would not do any harm to release an excess. If we consider a fly-belt covering 1000 square miles with the population density found by Jackson [6], there would be altogether 3.5×10^6 females, pupae and adults. On the optimistic assumption of Fig. 3, this would require 6.3×10^6 homozygotes to be released for eradication. To produce this number in one year a colony of 250 000 adults [10] would have to be maintained. At the present fly feeding rates that we use this would require a colony of 4000 rabbits, but about half this number or even less of goats might be adequate.

IRRADIATION EFFECTS ON RESPIRATION AND BLOOD DIGESTION IN THE TICK Ornithodoros tholozani AND THEIR IMPORTANCE FOR THE STERILE-MALE TECHNIQUE

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Abstract

IRRADIATION EFFECTS ON RESPIRATION AND BLOOD DIGESTION IN THE TICK Ornithodoros tholozani AND THEIR IMPORTANCE FOR THE STERILE-MALE TECHNIQUE. Soft ticks feed exclusively on blood at very long intervals. The ingestion of blood is accompanied by a manifold increase in oxygen consumption. This increase in metabolic rate is not affected by irradiation up to 16 000 R. Irradiation doses much lower than that required to produce 99% dominant lethals in males reduce the rate of digestion of blood taken by the tick. Thus at 4000 R the rate of digestion in males is slowed down, while 16 000 R is required to produce 99% dominant lethals. In females, a dose of 4000 R is necessary to prevent oögenesis, but the rate of digestion is slowed down even at 1000 R. This effect on digestion causes ticks which were fed immediately after irradiation to feed much less frequently thereafter, males and nymphs exposed to 16 000 R took a first blood meal after irradiation and did not feed again before death. Adult males are very little dependent on the blood meal for survival and successful mating, hence the irradiated males can be useful in controlling tick populations. Furthermore, since they do not take a second blood meal, they would not introduce any harmful effect into the environment.

1. INTRODUCTION

When developing a radiation sterilization method for the control of Ornithodoros tholozani (Laboulbène and Mégnin) ticks, the authors found that a dose of 16 000 R was necessary to produce 99% dominant lethals in males. Female ticks, however, were much more sensitive to sterilization by irradiation – after exposure to 4000 R no eggs were laid, while treatment with 1000 R slowed down oögenesis considerably [1].

Irradiated ticks seemed to retain the blood meal that they had taken after irradiation much longer than untreated ticks. Ticks require a blood meal to develop eggs. Therefore, the authors investigated the possibility that the higher sensitivity of females was due to the inhibitory effect of irradiation on blood digestion.

Since the inhibition of blood digestion can also be expected to affect the viability of the ticks, the role of this phenomenon in the control of ticks by the sterility method was studied.

2. MATERIALS AND METHODS

Ticks were reared according to a method described in a previous publication [1]. Virgin ticks were obtained by keeping blood-fed nymphs individually in vials and sexing them after their moult.

The rate of blood digestion was studied in the following manner. Ticks were weighed, allowed to feed on rats and then weighed again immediately after feeding, before there was any opportunity to excrete any coxal fluid. After weighing, each tick was placed in a separate vial upon which the amount of blood taken by the tick was recorded. At appropriate intervals the ticks were dissected, the gut content was washed into 5 ml distilled water and the debris and digested insoluble fractions were removed by centrifugation. The concentration of the haemoglobin in the supernatant was determined in a Beckmann spectrophotometer at 540 mμm. Since the amount of haemoglobin initially ingested by the tick was known, the percentage of digestion could be calculated. For each determination, three ticks were used.

Measurement of oxygen uptake was carried out using a Warburg constant volume respirometer, with cups of about 20-ml capacity. The respirometer contained in the central well 0.2 ml of 20% KOH solution absorbed on filter paper, and 0.2 ml water in the side arm. Ten ticks were placed in each cup and three groups were used for each determination. Measurements were carried out at 22°, 26° and 30° C. Readings were taken at 30-minute intervals for three consecutive hours. The measurements were repeated daily for the first two weeks, and then at weekly intervals.

For irradiation, a ^{60}Co source of the type Gammacell 200 was used, emitting 7000 R/min. For a dose of 1000 R, a 30-kCi ^{60}Co plate irradiator was utilized. The ticks were held in plastic petri dishes during exposure to the radiation.

For histological studies, ticks were fixed in Carnoy's fixative for three hours and cleaned in cedarwood oil overnight. Embedding in paraffin for 48 hours was necessary because of the large amount of blood inside the gut. Ticks were sectioned at 7 μm and stained in Giemsa.

3. RESULTS AND DISCUSSION

Adult ticks can survive for up to several years under conditions of starvation. During this period they maintain very low metabolism. In their natural habitat, the soil of caves, where the average temperature is about 22° C, the female tick requires less than 1 mm^3 O_2/h, which is less than 0.1 mm^3 O_2/mg live weight (Fig. 1). No eggs are produced by the female without a blood meal. When offered a rat, the female takes an average of 70 mg blood within less than 1 h.

This large blood meal causes an initial instantaneous increase in metabolism, followed by a continuous increase which reaches a peak within 8 - 12 days. At the peak, the respiratory rate is about 8-fold higher than that in the state of starvation (Fig. 1).

These observations differ from those reported by Kozanchikov [2] who found that O. tholozani displays a reduced oxygen demand after a blood meal. The respiratory pattern measured by the authors is very similar to the pattern showed by nymphs of Dermacentor marginatus and Ixodes ricinus, where an even greater increase in respiratory rate due to feeding was observed [3]. A considerable increase in metabolic rate after a blood meal was also observed in mosquitoes [4]. Belozerov claims that it is impossible to explain this marked increase in metabolism solely

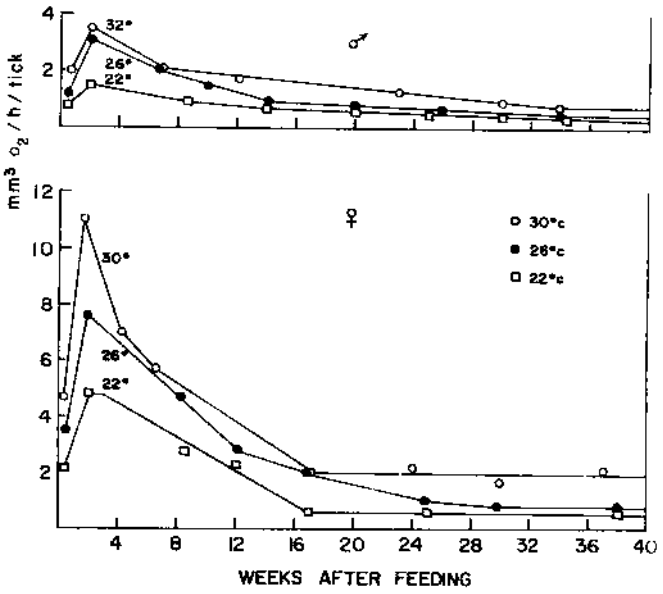

FIG. 1. Oxygen consumption by ticks

by the "dynamic effect of proteinous food". He attributed it to intensive growth and development of the integument, intestine, gonads and other organs which accompanies the prolonged feeding of the ixodid nymphs [3].

This explanation is quite inapplicable in the present case where adult females were used. In these females the main change following feeding is in the ovaries. Indeed, the peak of respiration corresponds nicely to the peak of oögenesis (Fig. 2) and, therefore, one tends to correlate the two phenomena.

However, when respiration of irradiated females was measured, the same increase in metabolism after feeding was observed (Fig. 3). Even a dose of 16 000 R, which causes complete degeneration of the ovaries, did not affect the pattern and the rate of respiration.

It thus seems that blood ingestion does have quite a strong 'specific dynamic effect'. If a non-nutritive solution consisting of 10^{-3} M glutathione in 0.15 M NaCl is offered to the ticks, they engorge it to the same extent as blood [5]; but this meal is not accompanied by any increase in oxygen uptake. Whether the increase is due to the blood proteins or to some other component remains unclear.

Further evidence that the increase in respiratory rate is independent of any developmental process can be found in considering the respiration of the males. Oxygen uptake increases 2 - 3 fold after a blood meal (Fig. 1), although no considerable development of any organ follows the meal. The increase is indeed much smaller than that found in females, but so is the amount of blood taken: an average blood meal of 11 mg as compared with 70 mg taken by the female.

In addition to triggering an increase in the metabolic rate, the ingestion of the blood meal starts a very strong digestive process. In females, over 70% of the blood proteins are digested within one month, and after 2 months

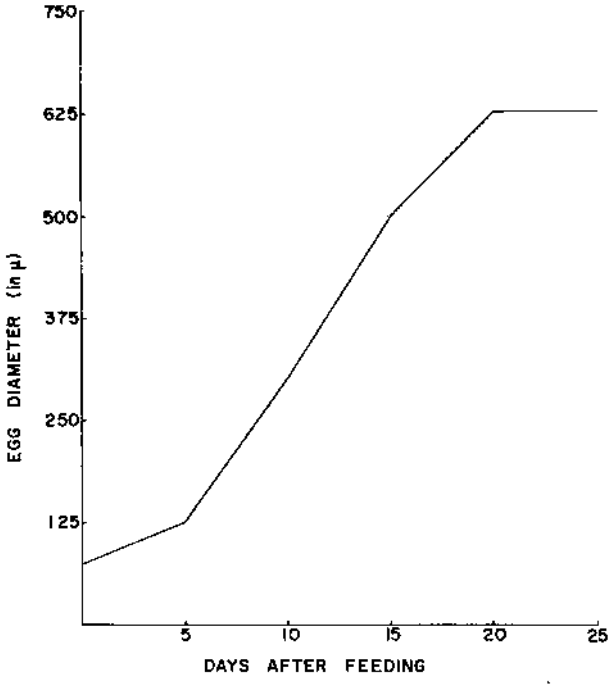

FIG. 2. Rate of oöcyte development

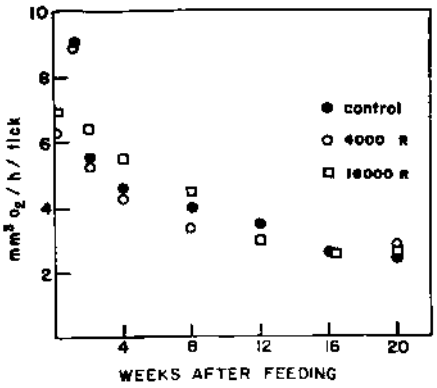

FIG. 3. Oxygen consumption by irradiated females

90% of the proteins have been utilized. At this stage, the rate of digestion decreases so much that the remaining 10% of undigested food can maintain the tick for months or years though at a very low metabolic rate.

This pattern of digestion seems to be quite wasteful. An adult tick weighing 10 mg consumes within two weeks over 7 mg protein, most of it being utilized as an energy source rather than for egg production. This point is illustrated in virgin females which show scarcely any oögenesis after a blood meal [6], but nevertheless digest over 50% of the blood proteins within one month (Fig. 4).

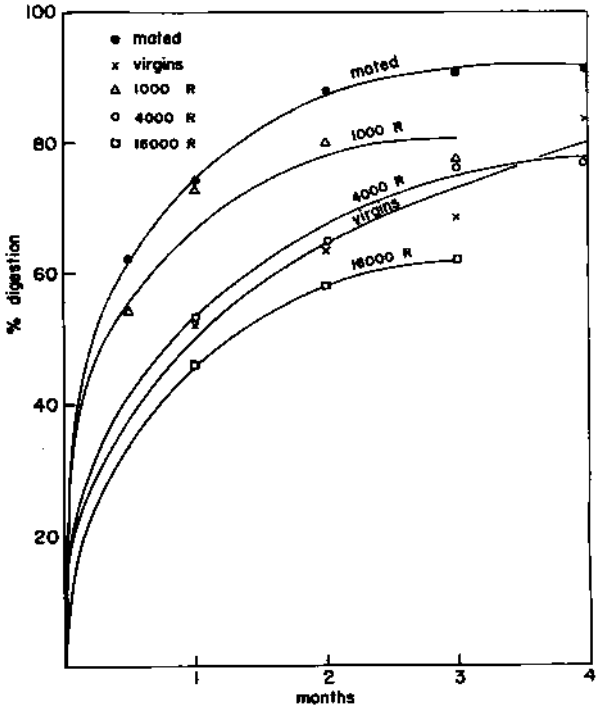

FIG. 4. Blood digestion in females

If the curves of digestion for mated and virgin ticks in Fig. 4 are compared, it can be seen that only about 20% of the blood proteins ingested by the ticks is utilized for egg production.

Thus the rate of digestion is regulated only to a small extent by the amount of proteins needed for the developing oöcytes. A major part of protein utilization not concerned with egg production is triggered by the blood meal, as can be seen from the increase in oxygen uptake and digestion which occurs in mated, virgin and irradiated ticks.

Further evidence for the role of the blood meal in triggering digestive processes independently of protein requirements is obtained from the pattern of blood digestion by the males. Although their rate of digestion is slower than that of the females, the males also utilize close to 70% of the blood meal within two months (Fig. 5).

Nymphs digest about 40% of their blood meal until they moult. Digestion continues at the initial rate for two months. During this period about 80% of the blood proteins are utilized. Here also the nutritional requirements of the body determine the rate of digestion only to a small extent. Nymphs exposed to 4000 R before the blood meal do not moult [1]. At this dose the rate of digestion during the first month is almost equal to that of the non-irradiated controls. Only in the second month does digestion slow down in the irradiated nymphs, indicating possible damage to the gut epithelium. After exposure to 16 000 R, digestion is slowed down in the nymphs from the first days after feeding (Fig. 6). At this dose survival is also considerably affected.

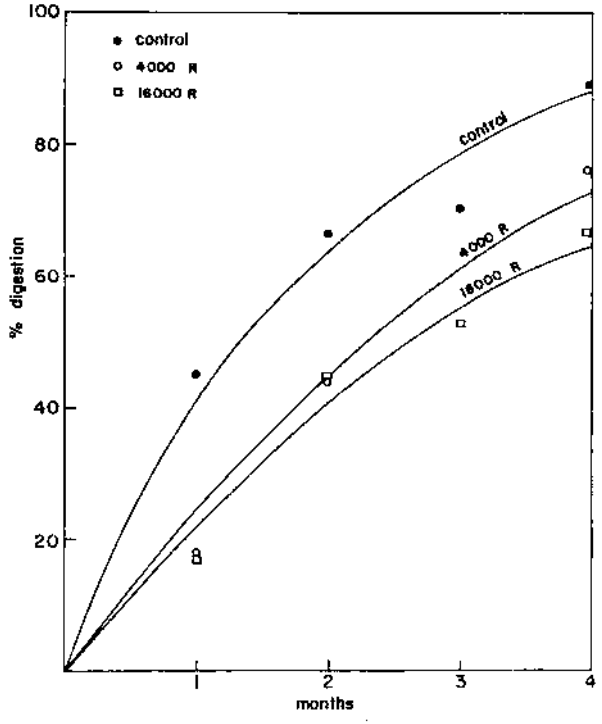

FIG. 5. Blood digestion in males

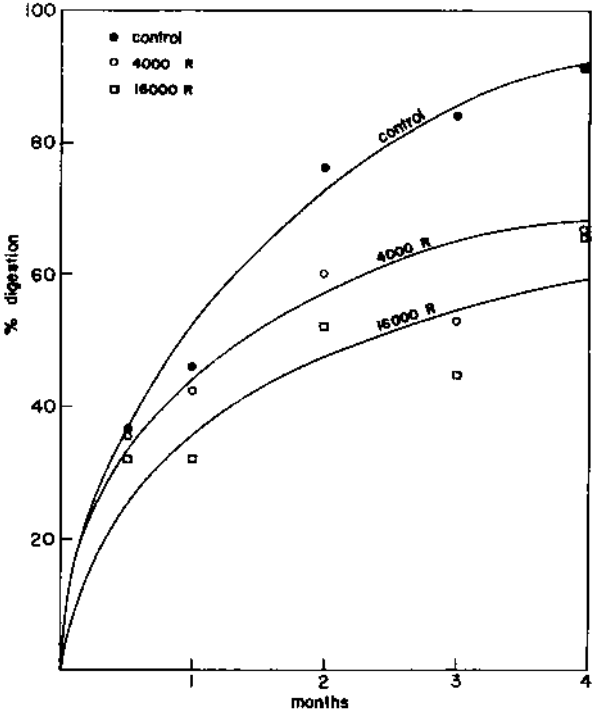

FIG. 6. Blood digestion in nymphs

The effect of irradiation on the rate of blood digestion in females was studied in more detail. It was found that, at a low dose of 1000 R, there is considerable inhibition of the digestive rate (Fig. 4). At this dose oögenesis is also slowed down. The average time required from feeding to oviposition is 75 days as compared with 23 days in the non-irradiated control ticks. It is difficult to distinguish whether oögenesis is retarded by the slower digestion rate or whether digestion is slowed down as a result of a feed-back mechanism regulated by the rate of protein uptake of the oöcytes.

In irradiated mosquitoes a delay in blood digestion was observed. The delay in this case was not due to a lack of proteolytic enzymes and could be related to egg development [7]. At low irradiation doses ticks also show some correlation between oögenesis and blood digestion. Females treated with 4000 R, which prevents oögenesis completely, digest blood at the same rate as virgins (Fig. 4).

Since the gut occupies most of the volume of the body, it is impossible to shield the gonads and irradiate only the gut, as was done with bees [8], or with the boll weevil [9]. Thus, a direct answer as to which of the two is the more sensitive tissue cannot be obtained. However, even if the gut epithelium is affected, the amount of digestion that still takes place after irradiation is quite high. Therefore, the primary reason for lack of development of the gonads at 4000 R could not be lack of the protein necessary for oögenesis. The higher sensitivity of females to sterilization by irradiation cannot be explained on this basis. At a dose of 8000 R (not shown in the figure) and at 16 000 R the rate of digestion was lower in the irradiated females than in untreated virgins – indicating here too possible damage to the gut epithelium.

To investigate this hypothesis further, preliminary histological observations were carried out, in which the gut epithelium following a blood meal was compared in mated ticks, virgins and ticks irradiated with 16 000 R.

When a female engorges blood the walls of the gut are strongly stretched. The stretched epithelial cells resemble a thin membrane with slight thickenings which contain the cell nuclei. After slow feeding the digestive cells start to enlarge. Three days after the blood meal the mated ticks show a few digestive cells reaching a diameter of 20μm. At this stage the virgin and irradiated ticks retain most of the epithelial cells in a resting state. The digestive cells continue to enlarge, so that in mated females seven days after the blood meal, the cells reach a diameter of 100μm. At this stage some of the cells bud off into the lumen of the gut. The floating cells, which assume a round shape, contain haematin particles. The haematin is the end product of the intracellular digestion of the ingested haemoglobin. This process is delayed in virgins and in irradiated ticks, whose digestive cells are only 30-40μm in diameter and contain no haematin; they are all still attached to the gut wall. Only 14 days after the meal does the digestive process of the virgins and irradiated ticks reach the stage arrived at by the mated ones on the seventh day.

All the digestive cells of the mated females contain many haematin granules 14 days after the blood meal. Many of the cells are already detached and float in the lumen of the gut. This stage is attained in the irradiated and virgin ticks only after 30 days.

The detached digestive cells are normally replaced by cells which differentiate from reserve cells. The reserve cells are smaller than the

digestive cells and their distal ends are usually covered by the growing digestive cells. Mitosis of the reserve cells is rarely seen in adult ticks. It is much more common in nymphs [10], the histology of which we have not yet studied. Additional histological studies are planned to determine the rate of replacement of digestive cells in irradiated ticks. It was found in Blabera that irradiation destroys the regenerative cells of the mid-gut, but does not damage the secretory cells which have already passed the differentiation stage, and which continue to function normally after irradiation [11]. It seems that in the female tick the differentiated digestive cells are also undamaged by irradiation. The slower rate of enlargement of the cells may be dictated by lack of development of the ovaries, since the same phenomenon was also observed in the virgins. It is suspected that the additional delay in the rate of digestion in irradiated females, which is seen several weeks after feeding, is due to damage caused to the reserve cells replacing the digestive cells.

We have seen that, to utilize sterile ticks for control, a dose of 16 000 R is necessary to produce 99% dominant lethals in males. This dose also destroys all the gonial cells, so that no recovery in fertility takes place. However, it is of great importance that the treated males maintain their vigour, longevity and sexual competitiveness. These seem to be affected to a certain extent by the irradiation [1], and this phenomenon may perhaps be attributed to the inhibition in digestion demonstrated in the males even after exposure to only 4000 R (Fig. 5). In this respect the male O. tholozani behaves similarly to the male boll weevil, where the irradiation dose producing a very high level of sterility is about 4 - 5 times that which produces damage to the gut epithelium [9]. Therefore, irradiated male boll weevils cannot be used for control purposes. Fortunately, unlike the boll weevil, O. tholozani males depend to a very small extent on a meal for survival and successful mating. Thus, in spite of the inhibition in the rate of digestion, more than 50% of irradiated male ticks survive for over 5 - 6 months, during which period they could be used for control purposes. Though they are not fully competitive, their effect on the extinction of the population is quite high [1].

Irradiation did not affect the percentage of ticks engorging immediately after the exposure. This is quite different from the behaviour of the female tick Amblyomma which does not engorge after exposure to 5000 R [12].

Since blood is digested more slowly by the irradiated tick, a much longer interval is required before a second blood meal is taken. Irradiated males which took a blood meal after treatment did not engorge again before death; nor did nymphs exposed to 16 000 R. However, about 30% of the irradiated females did take a second blood meal. Although this percentage is low when compared with the corresponding value of about 90% in the non-irradiated females, it is high enough to warrant separation of males and females before releasing the sterile males. Separation of the sexes is easy due to the big difference in size between male and female. Because of their smaller size, nymphs will contaminate the fraction which contains the males and will, therefore, be released together with them. The fact that males and nymphs do not take a second blood meal after irradiation makes their release into natural populations possible without adding any hazard to the environment. The only precaution required is to provide a meal before release.

REFERENCES

[1] GALUN, R., WARBURG, M., AVIVI, A., Ent. exp. appl. 10 (1967) 143.
[2] KOZANCHIKOV, I.V. Dokl. Akad. Nauk SSSR 32 (1941) 515.
[3] BELOZEROV, V.N., Ent. Obozr. 43 (1964) 288.
[4] GALUN, R., KINDLER, S.H., Science 147 (1965) 166.
[5] GALUN, R., Nature 185 (1960) 391.
[6] GALUN, R., WARBURG, M., Proc. Int. Symp. Insect Endocrines Brno (1966) in the press.
[7] AKOV, S., Ann. trop. Med. Parasit. 60 (1966) 482.
[8] LEE, W.R., J. agric. Res. 3 (1964) 113.
[9] RIEMANN, J.G., FLINT, H.M., Ann. ent. Soc. Am. 60 (1967) 298.
[10] BALASHOV, J.S. Parazit. Zb. 20 (1961) 185.
[11] MORTREUIL-LANGLOIS, M., C. r. Séanc. Soc. Biol. 154 1769.
[12] DRUMMOND, R.O., MEDLEY, J.G., GRAHAM, O.N., Int. J. Radiat. Biol. 10 (1966) 183.

DISCUSSION

V. NIGON: Your results on the effect of diet on respiration in ticks are very similar to those obtained at Lyon University on metabolic changes in insect larvae deprived of food. I refer to Fourche's work on Drosophila melanogaster, and that by Fourche and Coulon on Bombyx mori. Recent experiments confirm that this regulation of the respiratory metabolism is dependent on a hormone mechanism.

W.J. KLOFT: When determining the respiration quotient, Dr. Galun, did you measure CO_2 output as well as O_2 consumption? I ask this because of the continuous utilization of oxygen which is bound in large quantities to the blood erythrocytes in the gut, even when venous blood is used. The time for which the tick stores oxygen in its gut thus depends on the rate of breakdown of the erythrocytes during blood digestion. This oxygen may be absorbed by the body tissues or released through the gut, but in any case it remains in the working vessels and will influence your readings.

In Rhodnius prolixis, it seems possible to liberate all the oxygen from the haemoglobin relatively soon after feeding, which leads to excessive pressure in the vessels. Rhodnius has endosymbionts in the mid-gut which are sensitive to high oxygen pressure, and the insect therefore needs a blood deoxygenation mechanism. We are at present studying this problem at the University of Bonn.

Rachel GALUN: We attributed the strange results of our respiration quotient measurements to the fact that the CO_2 is released in large bursts rather than continuously. We did not consider the possibility of O_2 being bound to the blood haemoglobin.

K.K. NAIR: If you expressed your data on oxygen consumption per unit weight instead of per tick, would you find any difference between male and female? If the same were done after a blood meal, would there be any drop in O_2 consumption or would it remain more or less at a constant level?

Rachel GALUN: Females are only 1.5 times heavier than males and thus, per mg body weight, they take up more oxygen. After the blood meal, the true weight of the adult female does not change; one should not consider the blood stored in the gut as a part of the body weight.

H. MARCHART: Would you please specify in what way a second blood meal of your irradiated ticks, after release of the ticks into natural populations, would add any hazard to the environment?

Rachel GALUN: Through the irritating effect of biting by large numbers of ticks on the one hand, and the possibility of transmitting relapsing fever on the other.

P.A. LANGLEY: Is there no extra-cellular enzyme activity in the gut of the tick?

Rachel GALUN: No.

NEW SITE FOR THE ERADICATION OF A WHITE GRUB POPULATION (Melolontha vulgaris Fabr. AND Melolontha hippocastani Fabr.) BY RELEASE OF IRRADIATED ADULTS

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Abstract

NEW SITE FOR THE ERADICATION OF A WHITE GRUB POPULATION (Melolontha vulgaris Fabr. AND Melolontha hippocastani Fabr.) BY RELEASE OF IRRADIATED ADULTS. The area considered (about 1100 ha) is situated on the Swiss shore of Lake Constance and is infested with two cockchafer species (Melolontha vulgaris Fabr. and M. hippocastani Fabr.). The dynamics of the cockchafer population were followed from 1955 to 1967. Estimates of the cockchafer population were obtained by: (1) soil sampling for adults; (2) soil sampling for white grubs; (3) observation of flight activity and assessing damage on preferred food trees; (4) capture-recapture method with the aid of marked adults in spring 1967. Estimates were made several times with the isotopic dilution technique. Throughout the flight period 1967 the occurrence of adults was observed along the border of the Güttingerwald. Preferred food trees were regularly inspected and changes of preference with season were noted. Crepuscular flight activity of adults was watched at exposed corners of the wood. Changes in the flight direction during the period of observation were related to sex ratios in samples taken the following morning and compared with the developmental stage of the ovaries. Beetles were collected by shaking the host trees at dawn, and weight and volume of the samples were registered. Four therapeutical X-ray units potentially utilizable for irradiating beetles are available within 35 km. The nearest facility, 5 km away, was tested for its daily capacity.

1. INTRODUCTION

In a previously reported field trial carried out in north-western Switzerland (1958-1962) it was demonstrated [1, 2] that the sterile-male technique can successfully eradicate a white grub population. Since this field trial aroused interest among farmers and applied entomologists, a new site for the application of this technique was explored in north-eastern Switzerland.

2. DESCRIPTION OF THE REGION

The region is situated in the Canton of Thurgau on the Swiss shore of Lake Constance, some 8 km east of the town of Konstanz. The terrain includes the northern slope of a range of hills with altitudes ranging from 398 to 495 m above sea level. This range is topped by an extended wood called the Güttingerwald. Intensive general farming is practised in the whole area, with fruit trees, grass, cereals, potatoes, sugar beets, rape and green vegetables cultivated on most of the arable surface. Three predominantly rural villages, Altnau, Güttingen and Kesswil, are located between the Güttingerwald and Lake Constance (see Fig. 1).

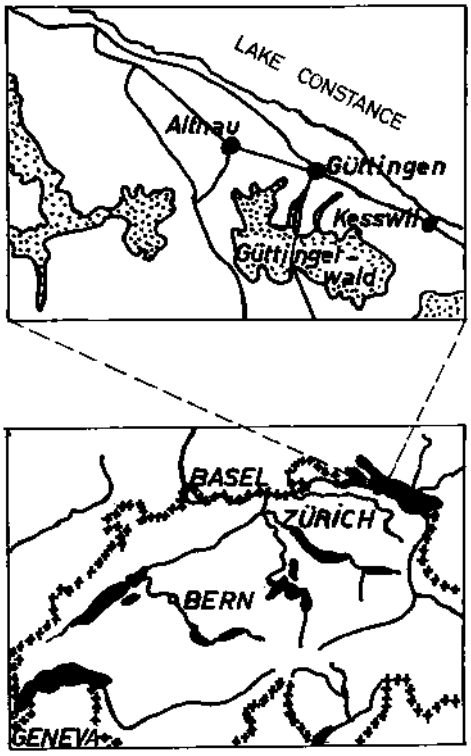

FIG. 1. Map of Switzerland showing the area explored for cockchafer (Melolontha sp.) eradication by the release of irradiated adults.

3. DELIMITATION OF THE INFESTED AREA

The infested area within the described region was outlined according to the method described by Schneider [3]. On 17 May 1967, when maximal defoliation of the oak trees appeared at the exposed borders of the woods, the boundaries of the areas most attractive to cockchafers were determined with the aid of an inclination meter, and these points were then fixed on a map (scale 1 : 25 000). The lines connecting these points formed the boundary around the infested area. This area measured 1128 ha including two villages, Altnau and Güttingen.

4. SURVEY ON POPULATION DYNAMICS

The region considered was infested with two cockchafer species, Melolontha vulgaris Fabr. and M. hippocastani Fabr. The flight period within the test area occurred simultaneously with that of the 'Uri cycle' (flight in 1967), whereas in the adjacent neighbourhoods to the east and west the flight occurred simultaneously with the 'Bernese cycle' (flight in 1966). Population dynamics were studied in connection with a survey

started in the central part of the Canton of Thurgau following an insecticide campaign against this pest in 1949 near Sulgen. In 1952 the surroundings of Altnau were included in this survey. From 1955 to 1967 this survey was continued every three years in the surroundings of the three adjacent villages of Altnau, Güttingen and Kesswil (see Tables I and II) and compared with several areas of the central part of the Canton of Thurgau.

TABLE I. COCKCHAFER POPULATION DETERMINED BY THE SAMPLING TECHNIQUE

Locality	1952	1955	1958	1961	1964	1967
Altnau	3.2	4.6	1.2	3.2	0.9	2.0
Güttingen	-	-	1.5	1.8	0.9	1.0

TABLE II. WHITE GRUB POPULATION DETERMINED BY THE SAMPLING TECHNIQUE

Locality	1952	1955	1958	1961	1964	1967
Altnau	8.9	2.4	6.2	1.4	5.2	15.3
Güttingen	-	6.6	5.8	1.8	1.3	5.5

5. SAMPLING TECHNIQUES

5.1. Soil sampling for adults

In autumn or spring before the flight started, the population density was estimated by digging and searching through sampling units of 0.25 m^2 of surface area down to a depth of 20 - 40 cm. From 4 to 11 plots of grassland of about 0.4 to 0.6 ha were chosen in each area. 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.

5.2. Soil sampling for white grubs

This procedure was repeated during late summer and autumn of the flight year. The sampling units were dug in the same plots as in the

previous surveys. For identification purposes the plots were numbered on a sketch-plan for each area and also on a 1 : 25 000 scale survey map for the whole region. The counts of cockchafers and white grubs served for the estimation of:

(a) the population density, sex ratio, and distribution of cockchafers just before the flight period;
(b) the population density and distribution of white grubs after the flight period;
(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.

5.3. Observation of flight activity

For the flight period during spring 1967, three points of observation were chosen at an exposed angle on the west border of the Güttingerwald, overlooking the surroundings of Altnau. Records were taken of meteorological conditions, of the number of flying beetles at 5-minute intervals and of the direction of their flight to or from the wood. These observations were made every evening at one point only, beginning on 8 May, one day after the first mass flight, until 24 May, when most of the females had returned from the first oviposition flight (see Table III). At the two other points observations were carried out irregularly.

5.4. Assessing damage on preferred food trees

At weekly intervals, beginning on 20 April, the borders of the Güttingerwald as well as those of the neighbouring woods were inspected for feeding by cockchafers. We inspected Quercus Robur L., Fagus silvatica L., Acer campestre L., Juglans regia L., Prunus cerasus L., P. domestica L., and Populus tremula L. which are preferred by cockchafers. On 21 June, when the last inspection tour was carried out, the oak trees were partially, and at several points almost completely, defoliated by Tortrix viridana L. The entire 13.2-km length of the border of the woods was classified as shown in Table IV.

5.5. Capture-recapture of marked adults

To estimate the size of the cockchafer population and to follow its dispersal, beetles were captured early in the morning from 9 to 13 May, 19 to 20 May and 23 to 24 May. A group of three men was equipped with a ladder, a hooked rod, a 4 × 4 m canvas and a series of wire cages and shook the trees to collect the beetles on the canvas and confine them in the cages. The captured beetles were transported to a cooling facility at the local farmers' cooperative. Samples were taken from each lot to determine the weight of the beetles, the sex ratio and the occurrence of the two species of Melolontha. After the release and recapture of marked beetles, individuals were inspected by a Raytech u.v. lamp Model LS-7

TABLE III. FLIGHT ACTIVITY AND SEX RATIO OBSERVED NEAR ALTNAU IN SPRING 1967

Date (in May)	Flight directed towards: wood	field	Sex ratio male/female	Observations on oviposition
8	2620	12	1.3	
9	2846	6	1.8	
10	2431	27	-	
11	1719	34	1.8	
12	536	273	2.1	Oviposition starts
13	387	1880	-	
14	266	1685	-	
15	12	3	-	
16	99	73	-	
17	452	40	-	
18	202	30	-	
19	1769	1852	3.1	Females return to the woods
20	214	109	1.9	
21	910	553	-	
22	1084	350	2.0	
23	-	-	-	
24	504	100	-	Increasing flight activity during daytime

TABLE IV. CLASSIFICATION OF DAMAGE ALONG THE BORDER OF THE WOODS

Damage classified as	Degree of defoliation	Length of the border (km)
Severe	Oaks completely defoliated, exposed beeches have 1 m of branches defoliated	4.2
Medium	Oaks 1/4 - 1/2 defoliated, exposed beeches have 1/2 - 1 m of branches defoliated	2.0
Slight	Oaks defoliated only at the ends of exposed branches	7.0
	Total length of inspected border	13.2

TABLE V. COCKCHAFERS CAPTURED IN A LIGHT TRAP

Date operated (in May)	Cockchafers trapped: males	females	Total	Marked individuals
18	-	84	84	1
19	44	322	366	2
22	77	25	102	-
24	10	65	75	1
Total:	131	496	627	4 = 0.64%

(Raytech Equipment Co., Somers, Conn., USA). Samples of 20 to 30 females were dissected to follow the development of ovaries and oviposition.

5.6. Marking of beetles with fluorescent dyes

The two dyes utilized for the bulk of the beetles were 'Nitrostella' Red and Yellow (Stella SA, Chatelaine, Genève). The red dye was based on BASF 'Lumogen LT feuerrot' and the yellow dye on BASF 'Lumogen

LT hellgelb'. From 9 to 11 May and on 18 May a total of 15 kg (about 15 000 individuals) of red-marked beetles were released near the central observation point. On 11 to 12 May and 17 to 18 May a total of 21 kg (about 21 000 individuals) of yellow-marked beetles were released at pt. 501.

A smaller lot of 1937 individuals handpainted with a white-bluish leather dye was released on 18 May near the third observation point, where a light trap was operated.

5.7. Captures in a light trap

A light trap was operated near the observation point A. Two bedsheets were fastened 4 m in front of two 125-watt mercury vapour lamps. Intercepted beetles were collected manually on both sides of the sheets (see Table V) from 18 - 19 May and on 22 and 24 May.

6. POPULATION ESTIMATES WITH THE ISOTOPIC DILUTION TECHNIQUE

At two observation points, the population was estimated by the isotopic dilution method. At the central observation point A, where red-marked beetles were released, the population density was higher than at the peripheral point B, where yellow-marked beetles were released (see Table VI). This estimate was compared with the daily flight observations and the damage assessment on preferred food trees.

The population estimate obtained by the isotopic dilution method for two selected points was extrapolated for the whole length of the 13.2-km border of the infested woods. This was done according to the degree of observed defoliation and classified in section 5.4. The cockchafer population within the delimited area was estimated to be between 5 1/2 to 25 1/2 million beetles (see Table VII).

6.1. Observation of flight behaviour

The fluctuation of the population estimates may be explained by the flight behaviour. The first oviposition flights began on 12 May. During the evenings of 17 to 19 May the direction of the main flight activity reversed, indicating that females were returning from the first oviposition. After 24 May flight activity gradually shifted to dispersal during daytime.

6.2. Sex ratio

From 12 to 19 May the ratio of male/female increased to 3.1 and then dropped to 1.9 (see Table III). This trend was observed for all observation points and was correlated with the behaviour of females as they returned to the population after oviposition.

TABLE VI. ESTIMATED COCKCHAFER POPULATION OF MARKED BEETLES AT TWO RELEASING POINTS

Releasing point	Period of observation (in May)	Individuals released	Marked individuals recaptured	Proportion marked individuals (%)	Correction factor	Population estimate
A						
Hangeten west	10	4032	15/2688	0.56	0.955	679 000
	11	8736	38/2520	1.5	0.965	550 400
north	12	6020	8/4512	0.18	0.93	3 100 000
	13	10820	36/2640	1.36	0.986	777 000
West + north	13	19556	64/2957	2.16	0.965	854 000
B						
Pt. 501	19	2327	28/4800	0.58	0.987	394 000
	20	7240	114/3020	3.78	0.928	169 500
	24	10832	79/4231	1.8	0.928	547 000

TABLE VII. ESTIMATED COCKCHAFER POPULATION ALONG WOODS IN THE INFESTED AREA

Example	Length of infested border (m)	Population estimate (isotopic dilution method)		Extrapolated estimate on total length of borders of woods		
		minimum	maximum	length (km)	minimum population	maximum population
Hangeten	600	550 000	3 100 000	4.2	3 740 000	21 100 000
Pt. 501	300	170 000	500 000	2.0	1 200 000	3 500 000
Horben	700	50 000	100 000	7.0	500 000	1 000 000
				13.2	5 400 000	25 600 000

6.3. Ovarial development

On 13 May three-quarters of the females captured in the morning and dissected had been ready to oviposit. On 23 May, 5% of the females were ready for the second oviposition.

6.4. Distribution of the two species (M. vulgaris Fabr. and M. hippocastani Fabr.)

M. hippocastani represented 3.3 to 13.6% of the cockchafers collected, and 1.5 to 4.2% at points A and B, respectively.

7. POPULATION ESTIMATES WITH THE SAMPLING TECHNIQUES IN THE FIELD

In spring 1967, we found an average of 2 cockchafers ready to emerge per m^2 at Altnau and 1 per m^2 at Güttingen (see Table I). Based on these figures the estimated population would be 17.5 million beetles within the delimited infested area of 1128 ha.

8. IRRADIATION OF BEETLES

Of four therapeutic X-ray units potentially available at different hospitals within distances of between 5 to 35 km from the infested area, the nearest one at the Canton Hospital Münsterlingen was tested for its capacity. Two 'Siemens Stabilivolt' were operated at 163 and 156 kV, 6 mA and 2 mm Al filtration. They produced 94 and 86 R/min with a distance of 30 cm between tube and subject. The radiation field was 15 × 19 cm. Two cardboard boxes of this size, each with a 6 cm layer

TABLE VIII. WEIGHT OF INDIVIDUAL COCKCHAFERS[a] IN THE CANTON OF THURGAU

Flight year	Number weighed	Males (g)	Number weighed	Females (g)
1958	249	1.0460	229	1.1913
1961	90	1.0716	86	1.2418
1964	13	1.2124	17	1.3393
1967	49	1.1192	38	1.3379

(a) Melolontha vulgaris Fabr. weighed before flight

of cockchafers or about 4 litres of beetles, were exposed at a time to 5000 R measured on top of the boxes. The choice of this dose was based on laboratory tests (1955 - 1958) and a field experiment (1959 - 1965) previously reported [1, 2]. Dose measurements taken on top, between and below the boxes were 94.3, 56.0 and 39.0 R/min. To assure homogeneous irradiation of the beetles, the position of the two boxes was reversed at half exposure time. A total of 60 litres or about 21 000 beetles were irradiated and released after being marked with fluorescent dyes as described in section 5.6.

9. HANDLING AND RELEASE OF BEETLES

The handling of cockchafers destined to be irradiated and released involved the following operations at various localities and times:

Operations		Localities	Time
Capture of Beetles		Field	Morning
Processing:	Cooling	Farmers' cooperative	Morning
	Determination of weight and volume	Field laboratory	Morning
	Sex ratio	Field laboratory	Morning
	Ovarial development	Field laboratory	Morning
	Separation of species	Field laboratory	Morning
	Marking	Field laboratory	Morning
	Packing	Field laboratory	Morning
Irradiation		Hospital	Afternoon
Release		Field	Evening
Observation of flight activity		Field	Evening

Cockchafers are robust and can easily be handled without being damaged. An individual weighs approximately 1 g, but weight varies with locality, year and sex (see Table VIII). The 1967 cockchafers were of average size and weight. An average of 338 beetles per litre was observed (range: 310 - 380) in cooled condition; the volume was greater when the samples were warmed and agitated.

During transportation beetles were left at about 4°C. Dry vermiculite or sawdust was filled into the boxes to absorb excessive moisture and excretions. Surplus beetles were kept in wire cages at the farmers' cooperative fruit cellar with +1°C and 96% relative humidity.

10. CONCLUSIONS

In the region surveyed the cockchafer population consisted predominantly of Melolontha vulgaris Fabr. Infestation was localized in an area

of 1128 ha bounded by the 13.2 km border of the wood. During flight these beetles aggregated on a stretch of 6.2 km of the borderline. The main concentration occurred on a length of 4.2 km, where oaks were completely defoliated in the first 10 days of flight.

The size of this population was estimated by two independent methods to be between 5 1/2 and 25 1/2 million and 17.5 million, respectively. The nearest irradiation facility was in a hospital 5 km from the infested area with two therapeutic X-ray units and had a daily output of 20 litres. This installation proved inadequate to treat this amount of beetles within one flight period. It would be necessary to provide a source with a capacity of 2000 - 3000 litres/day near the site or to sub-divide the area and treat the different sections in consecutive flight periods.

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REFERENCES

[1] HORBER, E., In Radiation and Radioisotopes Applied to Insects of Agricultural Importance, IAEA, Vienna (1963) 313-32.
[2] HORBER, E., Waldhygiene 6 6 (1966) 161-70.
[3] Schneider, F., Mitt. schweiz. Landw. 2 2 (1954) 17-34.

DISCUSSION

B. NAGY: Are there differences in the radiosensitivity of the two cockchafer species (Melolontha vulgaris and M. hippocastani)? This might present difficulties in the use of the sterile-male technique in the case of a mixed population of the two species. Also, from the data you obtained from light-traps, have you found any differences in light sensitivity

(a) of young and old chafers respectively, i.e. during the first and last part of the flight period, and

(b) between adults that have not yet fed and those that have?

E. HORBER: We set up an experiment to determine the dose level for sterilization of both species, but unfortunately this was not successful because of the ability of the beetles to detect X-rays. Some of the beetles managed to leave the phantom and to move out of the X-ray beam during exposure, and the remainder which received the planned dose was too small to represent the whole species. As a preliminary result we obtained a higher dose level for M. hippocastani. The results were hard to interpret, because after a sharp drop in hatchability at 3000 R,

a long tail of residual hatching at higher doses was observed; even at 12 000 R some larvae hatched, but further development failed.

The captures in the light-trap were less than those expected on the basis of previous trials. Females were more abundant, which was also in contrast to previous experience. As these results are not representative, I cannot really answer the question.

W.J. KLOFT: Your plan to store adult cockchafers at low temperature from one flight season to the next seems very promising, and could increase the efficiency of your method, which is at present unique in that it uses only natural populations. Can you store the insects both before and after feeding has started?

E. HORBER: No. It is easy to store cockchafers for more than a year at +1°C and more than 90% relative humidity, provided that they are still in diapause when collected, but they cannot be stored for more than a few weeks under these conditions if feeding has started. We are now trying to ascertain whether they can be stored if collected during the first flight after emergence, but before feeding.

EXPLORATORY STUDIES ON THE ERADICATION OF THE KOREAN PINE CATERPILLAR Dendrolimus spectabilis BUTLER BY MEANS OF RADIATION*

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Abstract

EXPLORATORY STUDIES ON THE ERADICATION OF THE KOREAN PINE CATERPILLAR Dendrolimus Spectabilis BUTLER BY MEANS OF RADIATION. Experiments to develop suitable artificial diets to rear the larvae of the Korean pine caterpillar Dendrolimus spectabilis Butler were carried out in connection with studies on eradication by the sterile-male technique. Several nutritient media were tested on larvae of various instar stages, and one was found to be most successful, rearing 2nd instar larvae without loss of body weight. Mortality at 30 days after artificial feeding was less than one-third. This medium consisted of sucrose, L-ascorbic acid, β-sitosterol, yeast hydrolysate, brewer's yeast, Butoben, choline chloride, residue of pine leaves and sodium glutamate. The composition of the pine leaves as well as of the larvae was analysed to obtain information on the nutritional requirement of the larvae. The larval metabolism of carbohydrate, sterol and protein was also studied. The radiosensitivity of the larvae of the 1st through 4th instar stages is dose-dependent and radioresistance increases with instar age. Doses of 100 krad or less did not produce mortality in the 4th instar, and could be used as the sterilizing dose for irradiation of older larvae or pupae. The number of eggs laid by a normal female mated with males irradiated with a dose of between 1.5 and 100 krad during the pupal stage did not change. The hatchability, however, was affected by irradiation of 15 krad or more where complete absence of hatching was observed. With 5 krad or less hatchability was not reduced from normal (55.8%) while 10 krad resulted in partial reduction. The sexual competitiveness of males irradiated with 15 and 25 krad was about one-half of the normal males and the hatchability of the eggs was reduced by about 50% to 37 and 31% respectively. It was concluded that the use of the sterile-male method in the eradication of the pine caterpillar is feasible if the method for mass rearing of the larvae is successful. Work in this connection is in progress.

1. INTRODUCTION

The pine caterpillar Dendrolimus spectabilis Butler is a serious destructive pest in Korean forests and the development of methods to control it is a task of some urgency, since the pine is one of the most important forestry products. The insect was first described by Butler in 1877 [1], and many reports have been published since then, mainly in Japanese and Korean scientific journals, on its entomological description [1 - 3], the damage it does to trees, its bionomical behaviour [4-6] and possible means of extermination and prevention. Though

* Experiments performed under IAEA Contracts No.277/RB, 277/R1/RB and 277/R2/RB during the period 1965 - 1967. Some of the results were presented at the Panel on Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests, held in Tel-Aviv, Israel, 17 - 21 October 1966.

many attempts have been made to control the insect, no really effective long-term method has as yet been evolved.

Since the eradication of the screw-worm fly by the sterile-male technique [7 - 11] on the island of Curaçao, many workers around the world have been applying this method to other insects, e.g. the fruit fly, the boll weevil, and the codling moth. This project is the first attempt to use this procedure for control of the pine caterpillar.

Basic knowledge of the pine moth indicated that it was feasible to control the insect by means of gamma radiation. The pest has one host and has short pupal (about 15 days) and adult stages. The latter stage is about 8 days, so that any radiation effect on fertility is soon evident. The egg period is 6 days and the larval stage is about 10 months. After the ecological characteristics had been reviewed, especially those concerning reproductive behaviour, the feasibility of applying the sterile-male technique to eradication of the insect by gamma radiation was examined. In view of the promising results of our previous experiments on radiation effects on the pine moth [12], we decided to develop an artificial diet for mass rearing of the insect from the larval stage, which could lead to successful application of the sterile-male technique. Due to the complexity of the problem and no information pertinent to artificial rearing, we analysed the composition of pine leaves which form the natural nutritional source of this insect. The radiosensitivity, the fertility and the sexual competitiveness of irradiated males were also studied.

2. EXPERIMENTAL PROCEDURES

2.1. Life cycle of the pine moth

Before and during the experiments, data gathered on the life cycle and behavioural aspects of the moth confirmed those published several decades ago [3, 8].

2.2. Radiosensitivity of young larvae

The larvae of 1st through 5th instars were collected (August and December) from the branches and bark (for hibernating 5th instars) of pine trees, and the larvae of each instar were divided into equal lots. In a large room (25°C, 60% relative humidity), screened against direct sun rays, each experimental lot consisting of 30 to 50 larvae was placed in a cylindrical vinyl mesh cage (height 60 cm, diameter 20 cm). To simulate the natural environment, fresh twigs of pine were continuously supplied in the cage. After a few days of rearing by this semi-artificial method, the larvae of each instar were again divided into nine experimental groups for irradiation, ranging from 0(control) to 600 kR. The irradiation was given by a ^{60}Co panoramic irradiator (capacity 1000 Ci, dose rate 2500 R/min, target distance 7 cm). The irradiated larvae were returned to the cage and reared as before. Mortality was recorded daily until 10 days after treatment.

2.3. Fertility of adult males irradiated during the pupal stage

The larvae of 7th and 8th instars were collected from April to July and reared as described. After the larvae became pupae, the males were separated from the females. At 10 to 13 days of pupal age, the males were divided into eleven experimental groups, each consisting of 40 to 80 pupae, for irradiation ranging from 0 to 100 kR. The irradiated male pupae were caged as before until emergence, which took place a week to ten days after treatment. Upon emergence, which occurred in late afternoon, an individual male adult moth was placed in a smaller cage containing a virgin female adult moth. Copulation was observed and a few days after the mating oviposition began. The eggs laid by the female on the twigs of the pine were collected daily in pyrex test-tubes (15×2.5 cm) and counted. After the collection was completed, the mouths of the test-tubes were covered by pieces of nylon net cloth and stored in the room. The per cent hatchability of the eggs in each experimental group was then determined.

2.4. Feeding experiment with the artificial diet

Pine-caterpillar larvae in various instar stages were collected from the local forest and divided randomly into experimental and control groups. During the experimental period, the larvae were housed in glass jars (15 cm in diameter, 14 cm high), or in vinyl net cages (20×20×20 cm), each of which contained one to twenty larvae, depending on the instar stage. The mouths of the jars were covered with nylon net cloth. All the artificial diets were given in the form of pieces of thin film spread over paraffin paper sheet to prevent the unnecessary evaporation of moisture from the diet. In the course of the feeding test, growth, vitality and other developments were observed. All the ingredients except L-ascorbic acid and choline chlorides were mixed with pine leaf distillate, and homogenated in distilled water, then autoclaved at 15 lb/in^2 for 20 min to kill the micro-organisms. The pH of the medium was adjusted to 3.1 with 20% acetic acid if necessary. After the mixture had been allowed to cool to about 50°C, L-ascorbic acid and choline chloride were added and mixed thoroughly. During the feeding experiments, the media were not allowed to dry. In further studies on rearing larvae of different instar stages, various modified media were tested, in which protein hydrolysates from various sources and bacterio- and fungi-static agents were supplemented or omitted.

2.5. Sexual competitiveness of the irradiated male pine moth

Irradiated (sterile) males, normal males and normal females were placed in a vinyl mesh cage (3×3×3 m) with ratios of 1:1:1 to 50:1:1. The hatchability of the eggs produced by the female was recorded. In this experiment, only two doses, 15 and 25 kR, were used.

To determine the amino acid requirement, the indirect method with glucose-U-^{14}C was employed [19], and the individual amino acid was identified and determined quantitatively according to the method of Stein and Moore [20].

3. RESULTS AND DISCUSSION

3.1. Observations on the life cycle of the pine moth

The developmental stages and their durations are illustrated in Fig. 1. The findings on morphological development and reproductive behaviour were in agreement with those from the comprehensive studies of previous authors [3, 13]. Most of the life cycle of the moth was covered by the larval (pine caterpillar) period, which, in turn, was divided into eight instar stages. About 200 days of the larval period, however, were spent in hibernation during winter months. The short life span of the adult moths of both sexes and the essentially monogamous nature of the female were both advantageous and disadvantageous. One of the disadvantages was that the average adult life span of 6 – 8 days made the repetition of an experiment, especially the fertility test, extremely difficult. The weather conditions during the experiment were also an unpredictable factor.

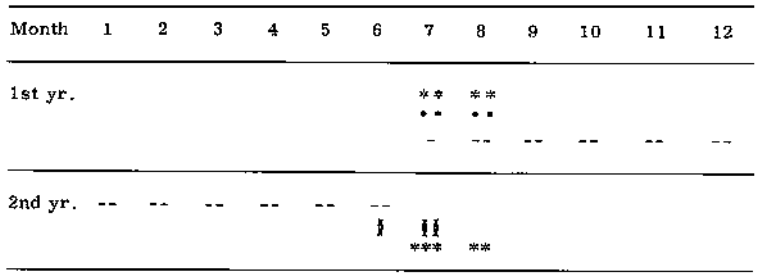

FIG. 1. Life cycle of Korean pine caterpillar Dendrolimus spectabilis Butler.
* Adult (life span av. 7 days; copulation soon after emergence)
. Egg (hatching in 10 days)
-- Larva (about 320 days)
1st instar: av. 10 days; 2nd instar: av. 18 days; 3rd instar: av. 15 days; 4th instar: av. 25 days; 5th instar: av. 160 days (hibernation): 6th instar: av. 16 days; 7th instar: av. 23 days; 8th instar: av. 34 days (End of the larval period identifiable by change of body colour from blue-red to black-brown, body length; male av. 7.8 cm, female av. 9.5 cm). Pupa (20 days), male smaller than female.

3.2. Radiosensitivity of younger larvae

Radiosensitivity in terms of mortality at 5 and 10 days after the irradiation of larvae is shown in Figs 2 and 3, respectively.

In each of the experimental instars (1st through 5th), mortality was dose-dependent and radioresistance increased with larval age. The difference in mortality between the most resistant (1st instar) and the least resistant (5th instar) larvae was considerable. At 5 days after irradiation (Fig. 2), for instance, a dose of 50 kR, which resulted in about 20% mortality in the 1st instar, produced no effect in the 5th instar. A mortality of 100% was observed at 175 kR in the 1st instar, but this dose did not influence the 5th instar. At 250 kR mortality of the 4th and 5th instars began to appear. An intermediate result was noted with both 2nd and 3rd instars, although the former always had higher mortality. Up to about 125 kR, these two irradiated instars showed slight differences in mortality,

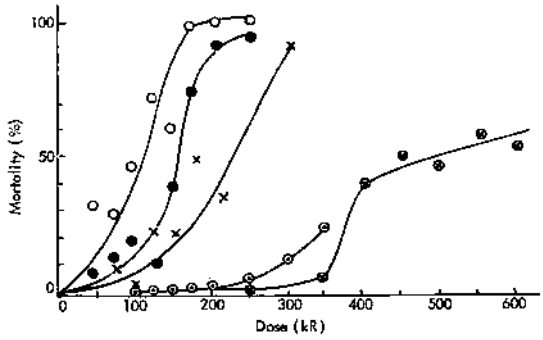

FIG. 2. Mortality of various instar stages of irradiated pine caterpillar larvae at 5 days post-irradiation o——o : 1st instar; •——•: 2nd instar; x——x: 3rd instar; ⊙——⊙: 4th instar; ●——●: 5th instar.

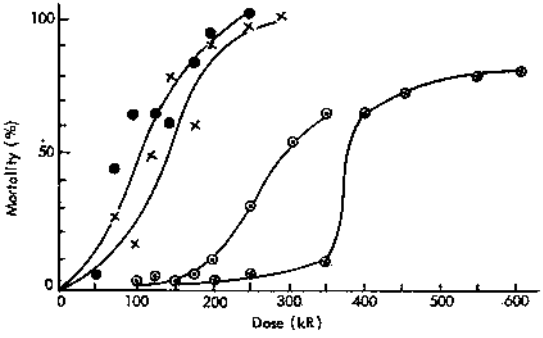

FIG. 3. Mortality of various instar stages of irradiated pine caterpillar larvae at various instar stages at 10 days post-irradiation; symbols are the same as in Fig. 2.

but above this dose mortality became greater and rapid: 90% in the 2nd instar at 200 kR, while the 3rd instar remained at less than 40%. In the 1st instar, with 250 kR, 100% mortality was obtained within 24 hours after the treatment. A loss of vitality usually preceded the death of the larva. In the later instar stages, the larvae were more radioresistant. A dose of 300 kR, which resulted in about 95% mortality within 5 days in the 3rd instar, produced no effect in the 5th instar (hibernating) larvae. However, with a dose of 400 kR remarkable increases in mortality of larvae were observed.

At 10 days post-irradiation, although the individual values of mortality naturally were higher than those at 5 days after treatment, essentially similar patterns of mortality were observed (Fig. 3). No result was available for the 1st instar because of high mortality before 10 days post-treatment. The mortality of the 2nd instar was higher than that of the 3rd instar at every dose applied, but the difference between them at higher doses was not so great, indicating a higher death rate of the latter between 6 and 10 days after the treatment.

When a comparison was made between the data of 5 days and 10 days post-irradiation, at doses less than 200 kR the mortality in the former was always approximately one-half that in the latter. This indicated that the

TABLE I. FERTILITY OF ADULT PINE-CATERPILLAR MALES GAMMA IRRADIATED DURING PUPAL STAGE

Dose (kR)	Number of pairs (RM × NF)[a]	Average number of eggs per female	Hatchability (%)
0	80	312	55.8
1.5	78	330	54.4
2.5	79	313	55.1
3	71	297	73.0
5	60	286	54.8
10	58	309	19.8
15	46	267	0
25	51	328	0
50	75	316	0
75	59	338	0
100	39	287	0

[a] NM: normal male, RM: irradiated male, NF: normal female

rate of larval death after irradiation was even and gradual during the first 10 days after treatment.

In view of the results presented here, it appears that the radiosensitivity of the hibernating larvae of pine caterpillars decreases as development progresses from the earlier instar stage to the later. For various species of insects, many investigators reported that young larvae were more radiosensitive than old larvae [14–15].

From the practical point of view, the prospect of using irradiated larvae in the fertility test was not an attractive one. Since doses of about 100 kR or less were not lethal for the 4th and 5th instar, 100 kR would be the maximum sterilizing dose applicable in the irradiation of pupae to produce irradiated adult male moths. The irradiation of eggs and larvae to produce sexually sterile moths was attempted, but many authors reported various undesirable side effects which made the oval or larval irradiation impractical [14, 16]. Doses ranging from 2.5 to 10 kR were applied to the larvae of the codling moth, the mallow moth and the cutworm moth.

3.3. Fertility of the irradiated male pupae

The average number of eggs laid by a normal female mated with a normal male was 312, as previously published [13]. In each group of irradiated males mated with normal females, regardless of dose between 1.5 and 100 kR, the average numbers of eggs produced by the female were not markedly different from normal (Table I). During the course of observations, no noticeable adverse effects were observed on the longevity of pupa or adult, on the copulatory and ovipositional behaviour in either

male and female, or on the vitality. The hatchability of the eggs in these irradiated groups showed a clear irradiation effect. The normal hatchability was 55.8% and, with doses of 5 kR or less, no reduction was observed. At doses between 15 and 100 kR, however, complete sterility of the male was noted in every irradiated group. The 10-kR irradiated group showed a significant reduction of hatchability (19.8%). At 3 kR, the 17% increase of hatchability without change in egg productivity was considered a stimulatory effect. Such an effect was found in the rate of emergence in the granary weevil [17].

The results suggested that the timing (later half of the pupal stage) of irradiation in this experiment to obtain sterile male moths was suitable because no serious side effect was noticed. Irradiation of pupae that were about 5 days old was not successful. The latest pupal stage was recommended as an effective irradiation time and applied to the bollworm [18]. Doses between 15 and 100 kR on the pupae in this experiment seemed adequate as sterilizing doses. Comparable effects were observed in the pink bollworm, the codling moth, the cutworm moth and the Colorado beetle with doses of 55, 40, 9 - 11, and 8 - 10 kR, respectively, [16, 18].

3.4. Sexual competitiveness of irradiated males

In this experiment, the hatchability of the eggs produced by normal males and normal females (1 : 1) was 48.24% (cf. values of 54.90% in 1966 and of 55.80% in 1965) which was not different from the previous experiment. When the ratio of normal males to normal females was increased to 2 : 1, about 10% reduction in hatchability was noted. This probably was caused by the harassment of the female by the males [9], which was undetected during observation.

When males irradiated with 15 kR were placed with normal males and females with the ratio 1 : 1 : 1, hatchability was 38.06% (the previous year's value was 37.5% with the same ratio) which is less than the normal value of 48.3%. Similarly, males irradiated with 25 kR with the same ratio further reduced hatchability to 36.3% (the previous year's value was 31.9%) (Table II).

Since theoretical hatchability for equal competitiveness of irradiated males to normal ones was 23.8%, the degree of reduction in hatchability of irradiated males in this experiment indicated that the sexual competitiveness of irradiated males was about half of normal. This finding agreed with many published data on various flies and on the pink bollmoth [18].

Thus, sterile males confined with normal males and females at various ratios (1 : 1 : 1 to 1 : 50 : 1) caused a reduction in the number of progeny. (This refers to the ratio of non-irradiated males to irradiated males to non-irradiated virgin females used in competitive mating tests.)

If one assumed an equal sex ratio in natural populations, males treated with 15 kR would be fully competitive when released in ratios of 1 : 30 : 1. In a mating competitiveness study on the horn fly, it was reported that effective sterility was obtained when the ratio of sterilized males to wild males was of the order of 20 : 1 [21].

TABLE II. EFFECT OF STERILE MALE RELEASES ON THE REPRODUCTIVE POTENTIAL OF THE PINE CATERPILLAR

Dose (kR)	The ratio of irradiated males to non-irradiated males to virgin females	Number of irradiated males tested	Total number of eggs laid	Number of eggs hatched	Hatchability (%)
	RM : NM : NF				
0 (control)	1 : 1	150[a]	23 736	11 450	48.24
	1 : 1 : 1	100	15 824	6 144	38.06
	5 : 1 : 1	250	12 423	2 842	22.89
	10 : 1 : 1	300	7 193	1 078	15.00
	20 : 1 : 1	300	3 156	125	3.98
	30 : 1 : 1	300	2 959	0	0
	50 : 1 : 1	300	601	0	0
25	1 : 1 : 1	100	20 041	7 280	36.32
	5 : 1 : 1	250	9 633	1 803	18.71
	10 : 1 : 1	300	9 255	702	7.58
	20 : 1 : 1	300	2 901	90	3.10
	30 : 1 : 1	300	1 632	0	0
	50 : 1 : 1	300	1 130	0	0

(a) Number of non-irradiated males

3.5. Rearing larvae on artificial media

3.5.1. Initial attempts to feed the larvae

To simulate the natural diet, fresh pine leaves were crushed and the residue was lyophilized after removal of the fat soluble fractions. The final diet was made by mixing this lyophilized fraction with leaf materials obtained by steam distillation (Medium 1). This medium was not successful; no larvae of the 3rd through 6th instar stages approached it. Next, a modification of an artificial diet for the silkworm [22] was used as a basal diet. To make the complete medium, vitamin and mineral mixtures and pulverized pine leaves were added to this basal diet. Although this was approached by the larvae of the 5th instar at the beginning, after about 5 days they stopped feeding.

It was found that the ascorbic acid content of the fresh pine leaves was relatively high, 0.4 mg/g of fresh leaf. In the third trial, the vitamin and mineral mixtures of Medium 2 were replaced by 40 mg of ascorbic acid. Although this was not successful for feeding 1st and 2nd instar larvae, a partial success was brought about with the 7th and 8th instars. Larvae which fed on Medium 3 weighed less than the control; nevertheless, about 40% of them pupated and formed cocoons. The onset of the pupal stage in the experimental larvae seemed slightly earlier and the cocoon was thinner and coarser than the control. These pupae died early. One reason for the difference in response to Medium 3 between the earliest and the latest stage larvae appeared, among other things, to be the inability of the young larvae to digest the casein. It was also felt that it might be useful to use casein hydrolysate instead of casein itself as the protein source. Other reports indicated that the ascorbic acid was a necessary nutritional requirement in the growth of insects [23]. The biting and swallowing actions of silkworm larvae were partly dependent on the agar contained in fresh mulberry leaves.

3.5.2. Studies on the composition of pine leaves and larvae

In view of the results of the foregoing initial experiments, the development of a suitable medium seemed to require extensive information on the composition of both pine leaves and larvae to understand the nutritional requirements of the larvae. The following experiments were carried out for this purpose: Pine leaves were taken from Pinus densiflora S et Z. The analytical methods of carbohydrates, lipids, protein and other substances are listed in Table III, and special procedures pertinent to the treatment of the pine leaves are also summarized.

3.5.3. The second feeding experiment with different media

From results of the foregoing experiment, and also from knowledge of artificial diets for a rice insect [24], the leopard moth [25] and the olive fly [26], a modified medium was produced as Medium 4. The results with this medium were not successful with the first instars. Many combinations of various amounts of the ingredient did not improve the result. Medium 7, in which distillate and homogenate of pine leaf residue and sodium glutamate were added (Table III), was only successful in rearing

TABLE III. COMPOSITION OF NUTRIENT MEDIUM FOR Dendrolimus spectabilis LARVAE

Ingredients	Medium No.7	Medium No.8
Sucrose	1.0 g	1.0 g
L-ascorbic acid	400.0 mg	400.0 mg
Yeast hydrolysate enzymatic [a]	1.0 g	1.0 g
Beta-sitosterol	2.0 mg	2.0 mg
Brewer's yeast, U.S.P. [b]	2.0 g	2.0 g
Potassium sorbate [c]	26.0 mg	26.0 mg
Choline chloride	2.0 mg	2.0 mg
Pine leaf distillate	15.0 ml	15.0 ml
Pine leaf residue	2.0 g	2.0 ml
Sodium glutamate [d]	20.0 mg	80.0 mg
Nipagin [e]		30 mg
Nipasol [f]		30 mg

Note: The pH of the medium was adjusted to 3.1 by the addition of acetic acid.

(a) National Biochemical Corporation, Cleveland, Ohio.
(b) National Biochemical Corporation, Cleveland, Ohio.
(c) Hoechst, Leverkusen, Federal Republic of Germany.
(d) Sigma Chemical Co., St. Louis, Mo.
(e) Methyl p-hydroxybenzoate, Merck Co., Darmstadt, Federal Republic of Germany.
(f) Propyl p-hydroxybenzoate, Merck Co., Darmstadt, Federal Republic of Germany.

the 2nd instar larvae by causing a gain in body weight closer to that of the pine leaf fed control and modifying the mortality of the experimental larvae. The appetite of larvae on this diet seemed encouraging, because they not only ingested the diet itself but also the paraffin sheet on which the diet was spread.

The partial success of the glutamate in the medium was not immediately evident. Glutamic acid, although not essential for most insects, occupies a central role in amino acid metabolism. This amino acid is required as a phagostimulant by the pine caterpillar.

As regards the protein sources, the commercially available protein hydrolysates, i.e. bacto-peptone, yeast enzymatic hydrolysate, soy enzymatic hydrolysate and amino acid mixture (16 crystalline amino acids) were supplemented in the present studies. The results showed that the casein was not a preferable protein for the larvae. Among the above protein enzymatic hydrolysates, yeast enzymatic hydrolysate was the most preferable protein hydrolysate under aseptic conditions [8 - 10].

Our recent experiments with glucose-U-^{14}C to determine the amino acid requirement revealed ten essential amino acids, i.e. tryptophan,

TABLE IV. DISTRIBUTION OF ^{14}C IN FREE AMINO ACIDS OF THE PINE CATERPILLAR FOLLOWING INJECTION OF GLUCOSE-U-^{14}C

Amino acids	Counts/min
Threonine	1036
Asparagine	245
Taurine	197
Glycine	183
Glutamic acid	138
Iso-leucine	103.6
Hydroxyproline	99
Serine	69.3
Alanine	14.8
Tyrosine	12.5
Proline	11.9
Arginine	10.3
Leucine	10
Cystine	7.2
Valine	5.3
Histidine	1.6
Methionine	0.6
Phenylalanine	0
Lysine	0
Tryptophan	0

lysine, phenylalanine, methionine, histidine, valine, leucine, arginine, proline and tyrosine. Alanine and cystine were also considered essential (Table IV).

Among the carbohydrates, sucrose could be the most suitable one for the larvae of the pine caterpillar. It was recently demonstrated in our laboratory that sucrose and maltose metabolizing enzymes were present in the larval gut.

As described in a previous progress report (supplement to the progress report, Contract No. 227/R1/RB, 1966), among the experiments conducted last year, we succeeded in rearing larvae with a synthetic diet called Medium 7. In the present feeding experiment, the larval medium (Medium 8) was modified by increasing glutamate and supplementing fungicides, i.e., Nipagin and Nipasol (Table III).

The larva-rearing experiments with Medium 8 were successful in modifying mortality as well as body weight of the larvae (Fig. 4). Different

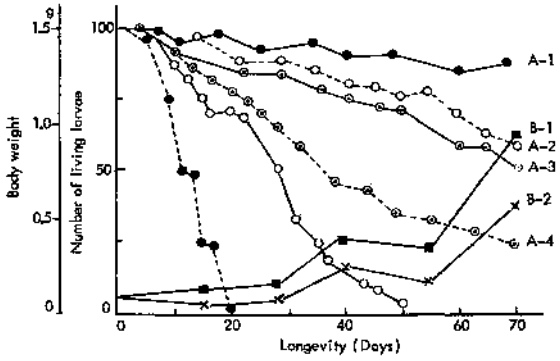

FIG. 4. Time course changes in the survival number and body weight of pine caterpillar larvae under various experimental conditions. A-1: fed on fresh leaves; A-2: fed on medium 8; A-3: fed on medium 8 (- yeast hydrolysate + amino acid mixture); A-4: fed on medium 8 (- yeast hydrolysate + soy hydrolysate); A-5: fed on medium 8 (+ yeast hydrolysate + blended carrot); A-6: fed on water only; B-1: body weight curve, fed on fresh leaves; B-2: fed on medium 8.

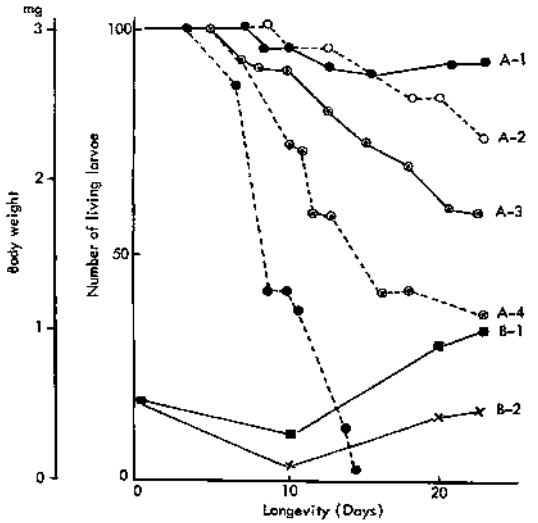

FIG. 5. Time course changes in the survival number and body weight of 2nd instar pine caterpillar larvae under various experimental conditions; symbols are the same as in Fig. 4.

combinations of formulae, changing the properties of each ingredient, were tested. Rearing experiments with Medium 8 on the larvae of 2nd and 3rd instar stages gave good results. The larvae gained body weight and modified the mortality (Fig. 5). The feeding experiment is continuing.

4. SUMMARY AND CONCLUSION

The radiosensitivity of the larvae of the Korean pine caterpillar D. spectabilis Butler, 1st through 5th instar stages, was dose-dependent,

and radioresistance increased with age. A dose of 100 kR or less did not produce mortality in the 5th instar and these doses could be used as the sterilizing dose in the eradication programmes.

Fertility and sexual competitiveness of the irradiated males were determined. Male pupae irradiated with 15 kR would be fully competitive when released in a ratio of 1 : 30 : 1.

Experiments were carried out to develop an artificial medium to rear larvae of the Korean pine caterpillar in connection with eradication by the sterile-male technique. Several media were tested with larvae of various instar stages. These media consisted of sucrose, L-ascorbic acid, yeast hydrolysate, beta-sitosterol, brewer's yeast, potassium sorbate, choline chloride, leaf distillate and residue and sodium glutamate.

Nutritionally essential and non-essential amino acids for the larvae were determined by the indirect method with glucose-U-^{14}C.

REFERENCES

[1] BUTLER, A.G., Ann. Mag. nat. Hist. 20 (1877) 481.
[2] UCHIDA, T.J., Fac. Agr. Hokkaido Imp. Univ. 25 (1930) 254.
[3] KAMIYA, K., Studies on the Morphology, Bionomics and Hymenopterous Parasites of the Pine-Caterpillar (Dendrolimus spectabilis BUTLER), Monograph publ. by Gov. of Korea (1934).
[4] EZAKI, T., Jap. Forest Bull. 16 (1922) 480.
[5] NAGANO, K., World of Entomol. 20 (1916) 489.
[6] SAITO, K.J., Forest. Soc. 13 (1933) 912.
[7] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 44 (1951) 725.
[8] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 46 (1953) 648.
[9] BAUMHOVER, A.H., GRAHAM, A.J., HOPKINS, D.E., DUDLEY, F.H., NEW, W.D., BUSHLAND, R.C., J. econ. Ent. 48 (1955) 459.
[10] KNIPLING, E.F., J. econ. Ent. 48 (1955) 459.
[11] LINDQUIST, A.W., J. econ. Ent. 48 (1955) 467.
[12] LEE, K.B., HWANG, U.K., PAIK, W.H., unpublished data, 1963.
[13] TAKAGI, G., Report of Forest Expt Station 2, publ. by Gov. of Korea (1925).
[14] PROVERBS, M.D., NEWTON, J.R., Can. J. Zool. 40 (1962) 400.
[15] HALBERSTAEDTER, L., GOLDHABER, G., HECHT, O., Growth 7 (1943) 413.
[16] LABRECQUE, G.C., KELLER, J.C., Advances in Insect Population Control by the Sterile-Male Technique, Technical Reports Series No.44, IAEA, Vienna (1965) 1.
[17] JEFFERIES, D.J., in Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 213.
[18] OUYE, M.T., GARCIA, B.S., MARTIN, D.F., J. econ. Ent. 57 (1964) 387.
[19] KASTING, R., McGINNIS, A.J., Can. J. Biochem. Physiol. 38 (1960) 1229.
[20] MOORE, S., STEIN, W. H. J. biol. Chem. 192 (1951) 663.
[21] HENNEBERRY, T.J., J. econ. Ent. 56 (1963) 279.
[22] FUKUDA, T., SUTO, M., KAMEYAMA, T., KAWASUGI, S., Nature (Lond.) 196 (1962) 53.
[23] ITO, T., Nature, (Lond.) 192 (1961) 951.
[24] ISHII, S., Symposium on major insect pests of rice at the International Rice Research Institute, Los Baños, Philippines September 14 - 18, 1964.
[25] MOORE, I., NAVEN, A., personal communication.
[26] HAGEN, K.S., SANTAS, L., TSECOURAS, A., Radiation and Radioisotopes Applied to Insects of Agricultural Importance, IAEA, Vienna (1963) 333.

DISCUSSION

G.W. RAHALKAR: I understand you performed your experiment with various sex-ratio combinations in insect cages. Would there be any change in your observed response if cages of larger size were used?

K.B. LEE: We have not experimented with cages of different sizes, and the number of insects per cage was always the same.

M. ANWAR: In Fig. 1 of your paper you mention that the life span of the adult is 7 days and in the same figure you give the oviposition period as 20 days. Could you please explain?

K.B. LEE: The period over which adults are found in the field ovipositing is about 20 days (mid-July to early August). The oviposition period of each adult is, of course, less than 7 days.

I.A. KANSU: Your Table I shows that hatchability of eggs is at its highest (73.0%) after 3 kR irradiation. Do you have any explanation of this figure (in the same experiment, control eggs show only 55.8% hatchability)?

K.B. LEE: In two sets of experiments out of three, we have observed a stimulatory effect on hatchability at this dose. Jefferies (Ref. [17] of our paper) has noted a similar effect in the irradiation of grain weevil pupae.

J.H.G. TICHELER: I note from your Table II that irradiated males are only one quarter as competitive as normal males. Did you try to improve their performance by lowering the irradiation dose, or by applying the irradiation at a later stage in the insects' development?

K.B. LEE: We irradiated the pupae at the latest possible stage. Tests with a lower dose are planned.

CRIA MASAL DE Dysdercus peruvianus G. Y SU ESTERILIZACION MEDIANTE RAYOS GAMMA

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Abstract — Resumen

LARGE-SCALE BREEDING OF Dysdercus peruvianus G. AND ITS STERILIZATION BY GAMMA RAYS. A method of large-scale breeding of Dysdercus peruvianus G. (family Pyrrhocoridae) with natural food and giving a hundred-fold population increase each month has been developed in the Entomology Laboratories of the Experimental Agricultural Station at La Molina, Lima, Peru.

Cages 90 x 60 x 60 cm are used; 2000 adults are placed in each, care being taken that there are not more males than females (two males to each three females are sufficient). The cage floor consists of wire mesh with nine strands per sqare inch; the roof, made of cloth or mesh with 14-18 strands per square inch, is fitted with a 15-W fluorescent light or a 60-W incandescent light, and the walls are of wood or some other washable material. Inside the cages are placed two containers with levant cotton shoots, and about 500 g of seed are scattered on the floor. Under the mesh is a sloping floor of plexiglas or some other smooth material which allows the eggs to slide down to a collecting channel ending in a tube marked in centimetres (1 cm^3 = 4250 eggs).

Similar cages, but with a wooden or brass floor, are used to contain ½ cm^3 of eggs, which are scattered on the floor covered with levant cotton seed. The eggs hatch and the nymphs grow in this cage until they reach the adult stage, when they are taken out in batches of 2000 and transferred to tanks for adults or irradiated.

Doses of 1000, 2500, 5000 and 10 000 R were used, and sterility was obtained from 2500 R, at which dose eggs were produced and development occurred to the first nymph stage. At 5000 and 10 000 R the eggs did not develop. At 1000 R development was normal, and in addition the cycle was accelerated and there were more eggs. Irradiated males and females accepted, and were accepted by, normal insects without discrimination. Competence levels of 2, 8, 32 and 128 males were tested.

CRIA MASAL DE Dysdercus peruvianus G. Y SU ESTERILIZACION MEDIANTE RAYOS GAMMA. Un método de cría masal de Dysdercus peruvianus G. (Fam.: Pyrrhocoridae) mediante alimentación natural, que permite centuplicar la población mensualmente, ha sido desarrollado en los laboratorios de entomología de la Estación Experimental Agrícola de La Molina (Lima-Perú).

Se emplean jaulas de 90 x 60 x 60 cm donde se liberan 2000 adultos procurando que el número de machos no sea superior al de hembras (dos machos por cada tres hembras son suficientes). El piso de las jaulas es de malla metálica de nueve hilos por pulgada cuadrada; el techo de tela o malla de 14 a 18 hilos por pulgada cuadrada está provisto de una luz fluorescente de 15 W (o incandescente de 60 W), las paredes son de madera u otro material lavable. Dentro de las jaulas se colocan dos recipientes con brotes terminales de algodonero y sobre el piso se esparcen unos 500 g de semilla. Debajo de la malla hay un piso inclinado de plexiglás u otro material liso que permite a los huevos deslizarse hasta una canaleta de recolección terminada en una probeta graduada en centímetros (1 cm^3 = 4250 huevos).

En jaulas similares pero con piso de madera o latón se pone ½ cm^3 de huevos que se esparcen en el piso cubierto de semilla de algodonero. Los huevos eclosionan y las ninfas se desarrollan en esta jaula hasta alcanzar el estado adulto, de donde se las extrae en grupos de dos mil y se las pasa a jaulas de adultos o irradiados.

Se probaron dosis crecientes de 1000, 2500, 5000 y 10 000 R, obteniéndose esterilidad desde 2500 R en que hubo producción de huevos y desarollo hasta ninfa I. Con 5000 y 10 000 R los huevos no se desarrollaron. Con 1000 R el desarrollo fue normal e inclusive el ciclo fue acelerado y el número de huevos mayor. Los machos y las hembras irradiados aceptaron y fueron aceptados, indistintamente, por los normales. Fueron probados niveles de competencia de 2, 8, 32 y 128 machos por pareja normal.

INTRODUCCION

El Dysdercus peruvianus G. (Fam. Pyrrhocoridae) está considerado como la plaga más perjudicial para el cultivo del algodonero en el Perú, pues los daños que ocasiona son de tal magnitud que provocan no sólo una reducción de la cosecha sino también una disminución de la calidad del algodón. En efecto, cuando el insecto ataca a frutos pequeños, o bien éstos caen y se pierden, o bien se paraliza su desarrollo o, finalmente, las semillas picadas dejan escapar aceite que mancha la fibra creando, además, condiciones favorables para el desarrollo de hongos que él mismo introduce con su trompa y que acaban por destruir la fibra.

Si a lo anterior agregamos que el insecto es migratorio, que no tiene enemigos naturales de importancia económica y que cada hembra es capaz de ovipositar 500 huevos, podremos comprender el peligro que representa el insecto.

Sumemos a ello que es resistente a todos los insecticidas clorados, que de los fosforados sólo el Parathion lo destruye, y ello mediante constantes y costosas aplicaciones de insecticidas, y que de los carbamatos sólo Sevin es empleado con éxito para combatirlo, pero que, en todos los casos las hembras oviplenas intoxicadas vacian su útero antes de morir y los huevos, que son fértiles, dan lugar a ninfas sólo después de 5 ó 6 días, cuando ya el poder del insecticida ha disminuido o desaparecido, y nos daremos cuenta de la necesidad de buscar nuevos métodos de combate.

Pues bien, esta chinche, conocida con los nombres comunes de "arrebiatado", rabiatado, culí-culi o chinche manchadora de la fibra del algodonero, ataca a la casi totalidad de las 200 000 hectáreas cultivadas con algodonero en el Perú, causando daños por varios milliones de soles, a los que hay que agregar los gastos que ocasiona la lucha contra el mismo.

IDENTIFICACION

Estos insectos pertenecen al orden Hemíptera, familia Pyrrhocoridae, géneros Dysdercus y Euryophthalmus, siendo las especies más comunes D. peruvianus G., D. ruficollis L., D. mimus Say., D. fernaldi Ballou, D. incertus Dist., D. rusticus Stal., D. imitator Blote y Euryophthalmus humilis Stal [1]. De ellas las tres primeras están distribuidas en toda la república, siendo la más abundante D. peruvianus G. En cambio, las otras cinco especies sólo se encuentran en la hoya Amazónica, no así en la Costa del Pacífico que es donde se cultiva el 95% del algodonero.

BIOLOGIA

Los huevos, de color blanco, son depositados sobre el suelo debajo de la hojarasca o bien en las comisuras de las bellotas (frutos) del algodonero, que comienzan su dehiscencia. Al cuarto o quinto día después de puestos, los huevos se tornan gradualmente amarillos y anaranjados y un día después de adquirir color anaranjado, eclosionan.

Las larvas recién nacidas son de color anaranjado, tono que se va intensificando a medida que pasan los días, hasta que al cuarto día mudan a su segundo estadio ninfal, cuando son de color rojo intenso. Este estadio dura de 4 a 7 días. Luego mudan y a partir del tercer estadio son de color rojo claro con líneas transversales blancas. Este estadio dura de 4 a 6 d. Hasta aquí las larvas o ninfas permanecen gregarias, pero al mudar a su cuarto estadio, que dura de 4 a 7 d, comienzan a separarse unas de otras y, finalmente, en su quinto estadio, que tiene una duración de 6 a 12 días, se movilizan en forma autónoma. Al final del quinto estadio las protuberancias alares son bien visibles y las ninfas se transforman en adultos.

Las chinches adultas tienen sus alas bien formadas y son de color amarillento, anaranjado y rojizo. Las hembras son más grandes y voluminosas que los machos, midiendo de 13 a 14 mm de largo por 3 a 4,5 mm de ancho. La maduración sexual, como es frequente entre los hemípteros, se alcanza en el último estadio ninfal, de tal modo que el mismo día que alcanzan el estado adulto y apenas se han endurecido las alas, se produce la cópula, que puede durar desde unas pocas horas hasta 4 ó 5 d. Normalmente una hembra copula de 5 a 7 veces en su vida y oviposita de 3 a 5 grupos de huevos. Una oviposición normal se compone de alrededor de 100 huevos, de donde resulta que cada hembra es capaz de poner entre 300 y 500 huevos. El porcentaje de recuperación (relación entre el número de huevos ovipositados y el número de adultos obtenidos de esa camada) es, en condiciones de laboratorio, de alrededor de 30%, lo que indicaría que el índice de reproducción de estos insectos está entre 1:90 y 1:150.

DAÑOS

Las ninfas, en sus tres primeros estadios, se alimentan de savia de las plantas y, a partir del cuarto estadio, son capaces de alcanzar y perforar la cáscara de las semillas alimentándose del aceite contenido en ellas. La fertilidad de las hembras está en razón directa a su alimentación con semillas.

Al perforar las bellotas para alimentarse causan lesiones tanto en los carpelos como en las semillas. De ambos escapan jugos que manchan las fibras.

Si la incisión se produce antes de los 20 días de edad de los frutos, éstos caerán o paralizarán su desarrollo; si ella se produce en cambio entre los 20 y 30 d, habrá producción de fibra pero ésta será dura y de inferior calidad. Finalmente, si la incisión se produce entre los 30 y 50 d de edad del fruto, éste sólo acusará el daño en el lóculo atacado, alcanzando los otros dos o tres su desarrollo normal; si ya el algodón está en franca dehiscencia, no habrá daño.

La razón de la importancia de los perjuicios que causa este insecto estriba no tanto en el daño directo, que acabamos de describir, sino en el daño indirecto que ocasiona al transmitir hongos patógenos, tales como Alternaria sp., Acremonium sp., Nematospora sp. y bacterias.

El arrebiatado no sólo ataca al algodonero sino también a plantas silvestres de las familias Bombacaceae, Malvaceae, Solanaceae y Amarylladaceae. Además puede vivir, sin causar daño, en plantas

cultivadas tales como el guayabo (Psidium guajava), el níspero (Mestilus germánica), la berenjena (Solanum melongena) y algunos cítricos.

Esto, unido a su capacidad migratoria, da al insecto una gran posibilidad de supervivencia a ún en las más variadas o adversas condiciones para su vida.

CRIANZA ARTIFICIAL DE Dysdercus peruvianus G.

Método Wille

El arrebiatado D. peruvianus G. es un insecto de fácil crianza en laboratorio, lo que explica que en el Departamento de Entomología de la Estación Experimental Agrícola de La Molina, Lima, Perú, se realice su cría desde 1926, cuando se creó el Departamento, y se mantenga actualmente una cría iniciada en 1930 por el Dr. J.E. Wille, sin que se hayan introducido, en los 37 años que ella se realiza, nuevos insectos. Debido a ello estos individuos son susceptibles a todos aquellos insecticidas a los que los individuos de campo han adquirido o desarrollado resistencia, por lo que tienen un inconmensurable valor, como testigos, en las pruebas de insecticidas [2].

El método de cría seguido por Wille consiste en recluir 100 a 150 adultos en un frasco de vidrio verde o azul de 30 cm de alto por 15 ó 20 cm de diámetro. Dentro del frasco, que se tapa con un pedazo de tela blanca amarrada al cuello del mismo, se pone un poco de arena en el fondo, un brote terminal de algodonero con dos o tres hojas y unas 20 ó 30 semillas (figura 1).

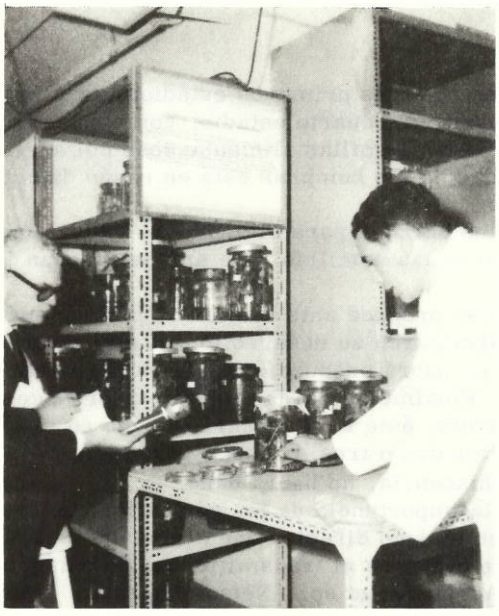

FIG. 1. Para la cría de D. peruvianus G. por el método Wille se emplean placas de Petri para guardar los huevos y los dos primeros estadios ninfales. Las ninfas III, IV y V, así como los adultos, se guardan en frascos de vidrio de boca ancha.

Los adultos se alimentan picando y chupando los jugos tanto de las hojas como de las semillas y las hembras ovipositan en la arena del fondo, entre las semillas.

Diariamente se cambia el brote y se sacan los huevos, los que se ponen en placas de Petri, con una hoja de algodonero fresca. Esta hoja se cambia cada dos días. Al quinto día los huevos blancos tornan a amarillo o naranja y al día siguiente eclosionan las ninfas que permanecen agrupadas sobre la cara inferior de la hoja.

Cuando las ninfas alcanzan su tercer estadio se las coloca en un frasco para adultos donde se les cambia el alimento y se limpia diariamente hasta que alcanzan el estado adulto [3].

La atención de cada placa de Petri toma de 2 a 3 min y la de cada frasco de ninfas de 4 a 8 min, dependiendo del número de individuos y la habilidad del operador.

En cada placa pueden guardarse hasta 500 huevos o ninfas y en cada frasco 500 ninfas o 200 adultos. De cada frasco de adultos se obtiene un promedio de 30 000 huevos, de los que se pueden recuperar entre 9 y 10 000 adultos en laboratorios mantenidos en condiciones naturales y empleando 12 h diarias de trabajo.

Disponiendo de seis frascos para adultos se puede obtener una producción diaria ininterrumpida de 2700 adultos o sea 81 000 mensuales empleando 40 horas diarias, 200 placas y 200 frascos.

CRIANZA MASAL DE Dysdercus peruvianus G.

Méthodo semiautomático

El Método Wille, si bien es seguro y sencillo, no permite producir, en forma económica, grandes cantidades de arrebiatados por lo que durante el año 1966 nos propusimos desarrollar un sistema de cría masal y económica, que creemos haber logrado en la forma que describimos a continuación.

En una jaula de 90 X 60 X 60 cm con sus cuatro paredes laterales construídas de madera u otro material lavable, el techo de malla plástica de 14 hilos por pulgada y el piso de malla metálica de nueve hilos por pulgada, se introduce, por cualquiera de las dos ventanas circulares de 15 a 20 cm de diámetro, que tiene una pared frontal, dos floreritos conteniendo agua y brotes terminales de algodonero cuyas bocas se han tapado con fibra de algodón a fin de que los insectos no se ahoguen y sostengan los brotes erguidos. Además se introducen dos platos de 15 cm de diámetro con semilla de algodonero y 2000 adultos de D. peruvianus G. sexados y en la proporción de dos machos por cada tres hembras (figura 2).

Debajo de la jaula hay un plano inclinado de madera, latón, plexiglás, o cualquier otro material liso que permite a los huevos, puestos por las hembras, rodar hasta una probeta graduada donde se recolectan y cuentan volumétricamente, tomando como unidad 4250 huevos por centímetro cúbico; sobre la jaula hay un foco de luz incandescente de 60 W o fluorescente de 15 W (figura 3).

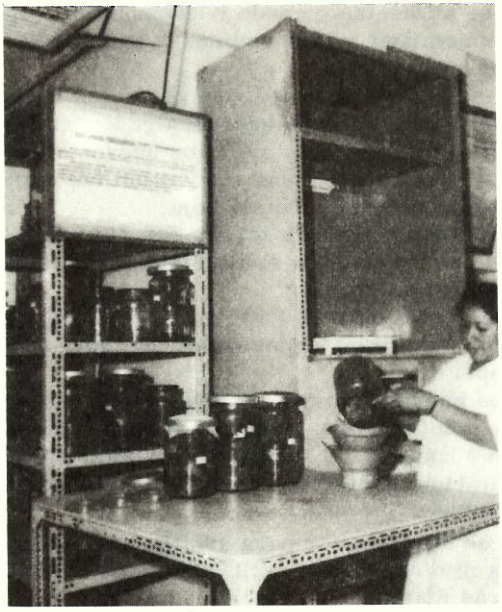

FIG. 2. Los adultos de D. peruvianus G. ovipositan en las semillas colocadas en el fondo de los frascos. Diariamente se extraen los huevos pasándolos por tamices.

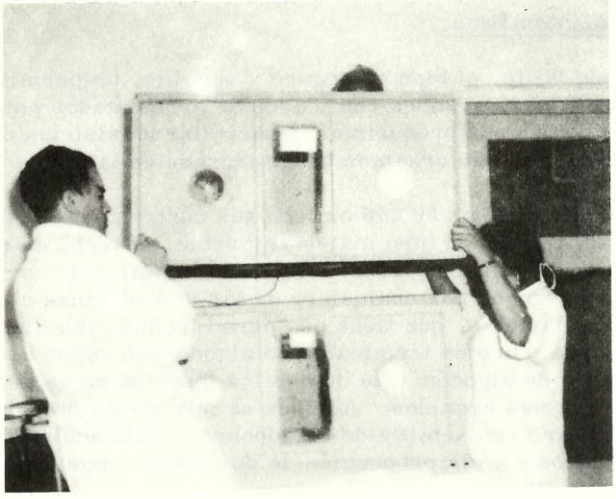

FIG. 3. En el método semiautomático de cría masal de D. peruvianus G. se emplean dos tipos de jaulas. La superior contiene los adultos y la inferior las ninfas.

Por cada jaula de adultos se deben tener 15 jaulas para ninfas, las que son iguales a las de adultos, con la única diferencia del piso que es también de madera o del mismo material lavable de las paredes.

En cada jaula para ninfas se introducen 5 cm^3 de huevos, de los que eclosionarán las ninfas que desarrollarán toda su vida dentro de la jaula

alimentándose de los terminales de algodonero, que se cambiarán cada dos días o a medida que sea necesario, y de las semillas de los platos. Diariamente, o cada vez que se cambien los terminales, se hará una ligera limpieza para retirar cadáveres y exhuvias.

Cada jaula de adultos produce, durante un período de 21 d contados a partir del sexto día de edad de los adultos, alrededor de 5 cm^3 de huevos diarios, con lo que se llenarán entre 15 y 20 jaulas para ninfas y, como la recuperación con este método es de alrededor de 50%, se producirán entre 150 000 y 200 000 adultos, o lo que es lo mismo 10 000 individuos diarios durante 20 días, a partir del mes de la primera oviposición.

Cada jaula requiere aproximadamente 4 min de atención diaria, alrededor de una hora para todo el sistema, lo que quiere decir que un solo hombre puede mantener ocho sistemas o, lo que es lo mismo, producir 80 000 individuos diarios. Es decir que, con las 40 horas diarias de atención que necesita el sistema Wille para producir 2700 individuos diarios, nosotros podríamos lograr, teóricamente, 400 000 individuos diarios.

ESTERILIZACION DE Dysdercus peruvianus G. MEDIANTE RAYOS GAMMA

Los trabajos se realizaron en el irradiador de ^{137}Cs, instalado en los Laboratorios de Entomología de la Estación Experimental Agrícola de La Molina, cedido por la USAEC por intermedio de la Junta de Control de Energía Atómica del Perú, teniendo como antecedente un trabajo similar ejecutado por el autor y el Ingeniero Godofredo García Baca, en 1961, en un irradiador de ^{60}Co durante la exhibición «Atomos para la Paz» [4].

Materiales y méthodos

El irradiador de ^{137}Cs de 20 000 Ci, doblemente encapsulado en acero inoxidable soldado en atmósfera de helio, tiene la fuente de irradiación colocado en una «jaula» que rodea la cavidad de irradiación, cuando está en posición de «irradiar». La irradiación externa promedio resulta así menor de 1 mR/h a 30 cm de la unidad.

La cámara de irradiación de sección pentagonal de 15 cm de diámetro por 20 cm de largo, está también construida de acero inoxidable y está equipada con puerta de clausura montada para deslizarse horizontalmente de la posición de «carga» a la de «irradiación» en menos de 4 s.

Un reloj eléctrico de 20 min, de programación automática, detiene la irradiación automáticamente al final del tiempo predeterminado de exposición. Para períodos más largos de exposición, se puede anular el reloj automático y proceder mediante control manual. Finalmente en caso que falle la corriente eléctrica se puede movilizar la cámara mediante un volante que se halla en un extremo del equipo.

Los insectos empleados fueron extraídos de las jaulas de crianza masal de Dysdercus peruvianus G., donde numerosas ninfas estaban mudando en adultos ese día, de tal modo que aún no habían copulado.

Los insectos fueron clasificados a simple vista (las hembras son más grandes y voluminosas que los machos) y confinados en la cámara

de irradiación, en grupos de 10 que luego de irradiados fueron confinados en frascos de vidrio, dándoseles una letra para cada intensidad de irradiación y un número para cada pareja, lo que se anotaba en el frasco respectivo.

Los frascos empleados fueron de vidrio opaco verdoso, de 20 cm de alto por 15 cm de diámetro, con boca ancha. Dentro del frasco se puso arena en el fondo y sobre ella un tubito con agua conteniendo un terminal de algodonero con tres hojas que se mantenía erecto gracias a un copo de algodón que a la vez tapaba la boca del tubito, evitando que los adultos se ahogaran en el agua. Además, se puso una cápsula de 4 cm de diámetro conteniendo semilla de algodonero. El frasco se tapó mediante una cubierta de tocuyo enmarcado en acero.

Diariamente se observaron los frascos y se anotó en un cuaderno los acontecimientos del día, tales como cópula, oviposiciones, número de huevos, muertos, número de ninfas de cada estadio, etc.

Los huevos fueron extraídos del frasco y colocados en una placa de Petri conteniendo una hoja tierna de algodonero. La placa se marcó con la letra mayúscula y número correspondiente al frasco, seguido de la letra minúscula «a» para la primera postura, «b» para la segunda, «c» para la tercera y así sucesivamente. La hoja se cambió diariamente. Si los huevos dieron ninfas éstas se mantuvieron hasta su tercer estadio en las placas y al cuarto estadio pasaron a un frasco preparado en la misma forma que el de adultos.

En un primer ensayo se irradiaron tanto a los machos como a las hembras, con las siguientes intensidades A:1000 R; B:2500 R; C:5000 R; D:10 000 R, y T:testigo normal sin irradiación.

En un segundo ensayo se irradiaron sólo los machos, excepto en el testigo, y se los confinó con una hembra normal. Las intensidades empleadas fueron: A:100 R; B:200 R; C:400 R; D:800 R; E:1600 R; F:3200 R; G:6400 R; H:12 800 R; y T:testigo normal, sin irradiación.

En un tercer ensayo se irradiaron con 5000 R y 10 000 R machos y hembras vírgenes, que luego se recluyeron, cada uno, con una pareja normal con el fin de conocer si el macho o la hembra irradiados eran rechazados por la hembra o el macho normales o si éstos tenían preferencia por los normales sobre los esterilizados.

Finalmente, en un cuarto ensayo se irradiaron machos con 10 000 R y se recluyeron con una pareja normal en las proporciones de 1:1:2; 1:1:8; 1:1:32; y 1:1:128, además de un testigo con 1: 129.

RESULTADOS Y DISCUSION

En los cuadros I y II presentamos los resultados obtenidos en el primer ensayo, cuando ambos sexos fueron sometidos a irradiación, y de su estudio puede deducirse, entre otras cosas, que la cantidad de huevos, aún cuando se produzcan, es inferior en las parejas irradiadas que en las normales. Se observa también que el desarrollo de los insectos fue prácticamente normal con 1000 R (tratamiento A); pero con 2500 R (B) sólo unos pocos huevos llegaron al estadio de ninfa I y luego murieron. Con 5000 R (C) y 10 000 R (D) hubo producción de huevos y aunque ellos no eclosionaron, ello nos indicó que la hembra del arrebiatado no fue esterilizada ya que, de acuerdo a las normas universal-

mente aceptadas, una hembra estéril no produce huevos; o bien, de acuerdo a los resultados obtenidos por el autor y García Baca en 1961, en que no se obtuvo eclosión de los huevos irradiados con 1000 R, 10 000 R y 100 000 R, la hembra no sería vírgen o ella sería capaz de

CUADRO 1. INFLUENCIA DE DOSIS CRECIENTES DE RADIACION EN LA VIDA DE Dysdercus peruvianus G. CUANDO AMBOS SEXOS FUERON IRRADIADOS (I.A MOLINA, PERU, 1967)

Pareja N°	N° de cópulas	Días de cópula	Oviposiciones	Huevos por postura	Longevidad ♀	Longevidad ♂	Días de Incubación	Intervalo entre posturas	Días por estadio I	II	III	IV	V	Relación
A-1	6	3	1	73	34	34	6	-	6	-	6	8	-	2:1
A-2	3	7	4	64	24	24	5	4	5	4	6	7	11	2:1
A-3	1	2	2	39	37	5	5	5	5	5	10	6	11	1, 5:1
A-4	7	3	6	76	30	42	5	5	4	6	6	8	13	2, 6:1
A-5	2	5	7	75	42	16	6	6	5	5	6	7	12	1, 6:1
X	4	4	4	66	33	34	5	5	5	5	7	7	12	1, 9:1
B-1	3	4	-	--	31	38	-	-	-	-	-	-	--	---
B-2	3	1	-	--	56	30	-	-	-	-	-	-	--	---
B-3	4	4	1	61	32	48	6	-	5	-	-	-	--	---
B-4	1	9	1	6	59	18	6	-	5	-	-	-	--	---
B-5	2	2,5	-	--	41	79	-	-	-	-	-	-	--	---
X	3	4	1	13	44	42	6	-	5	-	-	-	--	---
C-1	-	-	1	41	19	5	-	-	-	-	-	-	--	---
C-2	3	2	-	--	13	33	-	-	-	-	-	-	--	---
C-3	4	2	-	--	33	33	-	-	-	-	-	-	--	---
C-4	2	1	-	--	22	26	-	-	-	-	-	-	--	---
C-5	-	-	1	75	14	1	-	-	-	-	-	-	--	---
X	3	2	1	23	20	20	-	-	-	-	-	-	--	---
D-1	-	-	-	--	18	12	-	-	-	-	-	-	--	---
D-2	3	2	1	24	24	16	-	-	-	-	-	-	--	---
D-3	2	1	-	--	21	15	-	-	-	-	-	-	--	---
D-4	2	3	-	--	11	12	-	-	-	-	-	-	--	---
D-5	2	1	-	--	9	27	-	-	-	-	-	-	--	---
X	2	1	-	--	16	16	-	-	-	-	-	-	--	---
T-1	4	2	3	85	18	78	6	4	3	4	5	6	10	3:1
2	1	1	1	68	25	10	5	-	6	6	9	-	--	---
3	3	1	3	79	38	10	6	4	3	6	6	10	13	2:1
4	-	-	-	--	64	11	-	-	-	-	-	-	--	---
5	3	3	5	98	38	25	7	4	4	4	6	11	12	1, 5:1
X	3	2	3	82	37	27	6	4	4	5	6	9	12	2:1

CUADRO II. INFLUENCIA DE DIVERSAS DOSIS DE IRRADIACION EN LA VIDA DE PAREJAS ADULTAS DE D. peruvianus G.

Intensidad de irradiación	Nº de cópulas	Días de cópula	Oviposiciones	Huevos por postura	Intervalo entre posturas	Período de oviposición	Longevidad de los padres ♀	Longevidad de los padres ♂	Días de incubación	Duración de los estadios I	II	III	IV	V	Ciclo total
1 000 R	4	15	4	66	5	13	33	34	5	5	5	7	7	12	41
2 500 R	3	9	1	13	-	1	44	42	6	5	-	-	-	--	--
5 000 R	3	4	1	26	-	1	20	20	-	-	-	-	-	--	--
10 000 R	2	4	-	--	-	--	16	16	-	-	-	-	-	--	--
Testigo	3	5	3	82	6	9	37	27	4	4	5	6	9	12	40

producir huevos sin intervención del macho, en cuyo caso la irradiación de hembras y su liberación junto con los machos estériles no sería perjudicial.

En cuanto a los resultados del segundo ensayo que presentamos en los cuadros III y IV y V y en el que sólo los machos recibieron irradiación, podemos comentar que aparentemente la radiación, en intensidades inferiores a 10 000 R, afecta a los insectos obligándolos a realizar cópulas más prolongadas y disminuyendo el número de huevos puestos en cada lote, con la única excepción de los machos que recibieron 400 R, lo que podría deberse a mayor fecundidad de las hembras. También se pudo observar que la longevidad de los machos irradiados se redujo en un 88% para las bajas intensidades y en 66, 62 y 39% para las intensidades de 1600 y 3200, 6400 y 12 800 R, respectivamente, y que la incubación de los huevos es acelerada por las bajas intensidades y retardada por las altas, si bien es cierto que la duración del ciclo biológico no es aparentemente afectada por las bajas intensidades, pero sí es prolongada hasta casi en un 50% por las altas.

En el tercer ensayo las hembras y los machos irradiados con 5000 R o con 10 000 R copularon indistintamente con los machos y hembras normales, lo que indicaría que estas intensidades no reducen la capacidad de competencia de ninguno de los dos sexos.

En cuando a los niveles de competencia, si bien en este primer ensayo, en las proporciones de 32:1 y 128:1, los machos normales no encontraron a las hembras, creemos que debe repetirse la experiencia antes de poder señalar un nivel de competencia para este insecto.

CONCLUSIONES

1. Es posible criar en forma masal y económica el arrebiatado o chinche manchadora del algodonero, Dysdercus peruvianus G. en laboratorios acondicionados a 27°C, 5°C y 80% H. R. 5%.
2. La chinche manchadora de la fibra del algodón, D. peruvianus G. es fácilmente esterilizable con 5 a 10 000 R de radiación gamma.
3. La longevidad de los machos esterilizados mediante rayos gamma se reduce hasta un 61% en relación con la de machos normales.

CUADRO III. REACCION DE MACHOS DE D. peruvianus G. A BAJAS DOSIS DE RADIACION GAMMA

Pareja Nº	Nº de cópulas	Días de cópula	Oviposiciones	Huevos por postura	Intervalo entre posturas	Longevidad ♂	Longevidad ♀	Días de incubación	Días por estadios I	II	III	IV	V	Relación
A-1	5	10	3	45	9	37	25	5	5	5	5	5	9	2:1
2	3	5	2	88	13	37	27	6	5	6	6	5	10	2:1
3	5	9	3	42	7	38	59	5	4	5	4	3	9	2:1
4	5	16	4	47	3	37	59	8	3	5	5	5	8	2:1
5	7	16	3	35	5	37	45	5	5	5	5	5	9	2:1
6	5	16	4	83	6	37	59	8	4	5	5	5	7	2, 6:1
X	5	12	3	57	7	37	48	6	4	5	5	5	9	
B-1	7	22	2	52	19	43	59	5	3	5	4	5	8	2, 5:1
2	5	11	2	42	14	34	59	5	4	5	4	6	6	2:1
3	3	12	3	70	7	28	43	6	5	6	5	4	8	2, 6:1
4	6	14	4	72	3	34	35	6	4	5	5	5	8	2:1
5	8	26	2	60	27	42	55	6	5	6	5	7	8	1:1
6	6	17	4	35	4	34	35	4	4	6	5	5	8	1:1
X	6	17	3	55	12	36	48	5	4	6	5	5	8	
C-1	5	12	1	116	--	42	59	7	4	4	5	6	9	2:1
2	3	7	1	105	--	34	18	3	4	5	5	4	8	1, 4:1
3	6	18	1	58	--	36	37	7	3	6	5	5	7	1, 4:1
4	4	12	4	77	5	33	59	6	4	5	5	4	8	1, 3:1
5	3	7	1	115	--	28	59	7	5	6	5	5	20	1:0
6	7	22	3	62	10	40	42	6	4	6	6	5	9	1, 6:1
X	5	13	2	69	8	36	46	6	4	5	5	5	10	
D-1	7	14	3	84	4	36	59	4	4	5	5	5	8	1, 2:1
2	8	18	2	103	7	34	51	8	6	5	5	5	9	1:1
3	5	14	2	46	8	31	35	8	5	7	6	5	10	1:1
4	2	4	-	--	--	21	42	-	-	-	-	-	--	---
5	3	11	1	79	--	20	59	9	4	5	5	6	11	5:1
6	6	13	3	55	7	29	34	6	4	5	6	5	8	1, 5:1
X	5	12	2	81	4	29	47	6	4	5	5	5	8	
T-1	6	11	2	56	7	30	59	6	4	5	6	5	8	1, 9:1
2	4	14	3	66	7	38	59	6	5	4	5	5	9	3:1
3	6	14	3	84	8	40	41	5	4	6	5	5	8	3, 6:1
4	5	10	4	68	6	35	59	6	4	5	4	5	8	1, 6:1
5	2	2	1	79	--	23	40	9	4	4	5	5	8	---
6	5	11	2	79	4	44	59	8	4	5	5	5	9	1:1
X	5	10	3	72	5	35	53	7	4	5	5	5	9	

CUADRO IV. REACCION DE MACHOS DE D. peruvianus G. A ALTAS DOSIS DE RADIACION GAMMA.

Pareja N°	N° de cópulas	Días de cópula	Oviposiciones	Huevos por postura	Intervalo entre posturas	Longevidad ♂	Longevidad ♀	Días de incubación	Días por estadio I	II	III	IV	V	Relación
E-1	7	20	3	48	7	33	42	7	4	5	5	5	9	2:1
2	3	13	1	46	--	33	34	7	4	5	5	4	7	--:-
3	2	8	2	77	8	23	36	8	5	5	5	5	9	2,2:1
4	4	14	3	74	4	21	21	9	4	4	5	5	8	1,3:1
5	3	6	2	42	8	33	30	9	5	5	4	5	8	0:1
6	6	16	2	90	3	34	59	10	5	6	6	5	9	1,3:1
X	4	13	2	62	5	30	35	8	5	5	5	5	8	
F-1	8	15	3	60	3	33	31	18	5	5	4	5	8	2:1
2	1	5	3	40	6	49	34	5	3	5	5	5	8	1:2
3	5	15	2	55	8	42	34	27	5	5	5	7	7	1:1
4	7	13	2	72	8	33	35	6	4	4	5	5	8	1,2:1
5	1	3	1	53	--	34	25	6	5	5	4	5	7	1:1
6	4	15	1	91	--	33	49	7	5	5	5	4	9	1,5:1
X	4	11	2	64	4	37	35	12	5	5	5	5	8	
G-1	6	16	2	74	8	39	34	7	5	6	5	5	5	--:1
2	4	11	2	78	8	27	30	26	4	5	5	6	7	1:0
3	6	22	2	70	19	35	34	20	5	6	4	-	--	--:1
4	-	--	2	76	1	59	33	31	-	-	-	-	--	--:1
5	5	19	3	52	10	30	29	21	5	5	6	5	10	1:1
6	5	13	3	83	10	42	40	22	4	5	7	8	9	2:0
X	4	13	3	72	9	39	33	21	4	5	5	4	5	
H-1	2	6	2	67	4	17	16	33	-	-	-	-	--	--:-
2	1	1	4	54	6	56	36	39	-	-	-	-	--	--:-
3	1	7	3	42	7	59	22	30	-	-	-	-	--	--:-
4	2	5	3	83	9	33	15	-	-	-	-	-	--	--:-
5	-	--	-	--	--	11	25	30	-	-	-	-	--	--:-
6	1	5	1	96	--	15	15	7	5	5	5	5	7	1:1
X	1	4	2	57	4	30	21	23						
T-1	2	11	2	58	7	30	59	6	4	5	6	5	8	1,9:1
2	4	14	3	66	7	38	59	6	5	4	5	5	9	3:1
3	6	14	3	84	8	40	41	5	4	6	5	5	8	3,6:1
4	5	10	4	68	6	35	59	6	4	5	4	5	8	1,6:1
5	2	2	1	79	--	23	40	9	4	4	5	5	8	--:-
6	5	11	2	79	4	44	59	9	4	5	5	5	9	1:1
X	5	10	3	72	5	35	53	7	4	5	5	5	8	

CUADRO V. INFLUENCIA DE DOSIS CRECIENTES DE RADIACION GAMMA SOBRE LA VIDA DE MACHOS DE D. peruvianus G.

Intensidad de irradiación	N° de cópulas	Días de cópula	Oviposiciones	Huevos por postura	Intervalo entre posturas	Período de oviposición	Longevidad de los padres ♂	Longevidad de los padres ♀	Días de incubación	Días por estadio I	II	III	IV	V	Ciclo total
100 R	5	12	3	57	7	15	37	46	6	4	5	5	5	9	34
200 R	6	17	3	55	12	16	36	48	5	4	6	5	5	8	33
400 R	5	13	2	89	3	7	36	46	6	4	5	5	5	10	35
800 R	5	12	2	61	4	7	29	47	6	4	5	5	5	8	33
1600 R	1	13	2	63	5	7	30	35	8	5	5	5	5	8	36
3200 R	4	11	2	64	4	6	27	36	12	5	5	5	5	8	40
6400 R	4	13	3	72	9	14	39	33	21	4	5	5	4	6	44
12800 R	1	4	2	57	4	11	30	21	23	5	5	5	5	7	50
Testigo	5	10	3	72	5	11	35	53	7	4	5	5	5	8	34

4. Las hembras de D. peruvianus G., irradiadas hasta con 12 800 R, son capaces de ovipositar, aunque sólo en un caso los huevos eclosionaron.
5. Dosis entre 1600 R y 12 800 R prolongan considerablemente el ciclo biológico de D. peruvianus G.

REFERENCIAS

[1] WILLE, T., J.E., Entomología Agrícola del Perú (2a. Ed.), Junta de Sanidad Vegetal, Lima, Perú (1952).

[2] SIMON, F., J.E., Resistencia del arrebiatado de Dysdercus peruvianus G. a los insecticidas orgánicos, Inf. Men. N°368, E.E.A. La Molina (Marzo 1958).

[3] OHOSHI, J., SIMON, F., J.E., Determinación de las dosis letales del Sevin para Dysdercus peruvianus G. en strains resistentes y susceptibles al BHC, La Molina (1963).

[4] SIMON, F., J.E., García B. G., Efectos de la radiación gamma en el arrebiatado, Bol. de la Soc. Ent. Agr. del Perú, 3 (Junio 1963).

DISCUSSION

W.J. KLOFT: What daily photoperiod did you use in your mass rearing of Dysdercus peruvianus? We found from our experiments with related Heteroptera that 16 hours of light to 8 hours of dark was the optimum for development and reproduction.

J.E. SIMON: We illuminated the insects for 14 hours daily. Despite the fact that D. peruvianus is quite able to live all year round under Peruvian conditions, we discovered that a daily exposure of more than 12 hours of light significantly improved the reproduction rate.

W. J. KLOFT: Did you encounter the problem of mutual cannibalism in your crowded mass rearings? Even seed-feeding bugs like Oncopeltus sometimes show a tendency towards this pattern of behaviour.

J.E. SIMON: Well, we used the mesh at the bottom of the adults' cage expressly to avoid any damage to the eggs, and despite there being 2000 adults per cage we did not observe any cannibalism.

W.J. KLOFT: Are you sure that it is necessary to give the insects the additional food supply of cotton leaves? We found that our seed-feeder Oncopeltus fasciatus needed no green plant component in its diet, but could be reared on seeds and water only. This might prove a means of reducing the cost of your mass rearings.

J.E. SIMON: In fact, during the eight weeks previous to my visit to Vienna we were not allowed to crop the cotton leaves by virtue of our Plant Quarantine Regulations and so we have had to rear the bugs on seed and water only. I had feared that on return to Lima I would find that my culture of Dysdercus had perished, but your remarks have given me some encouragement to believe that this diet will be adequate, after all.

APPLICATION AUX *Oryctes* (COLEOPTERES: SCARABAEIDAE) DE LA METHODE DU LACHER DE MALES STERILES*

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Abstract — Résumé

RESEARCH INTO THE APPLICATION OF THE STERILE-MALE RELEASE TECHNIQUE TO THE RHINOCEROS BEETLE (COLEOPTERA:SCARABAEIDAE). The control of the Rhinoceros beetle, which causes considerable damage in palm and coconut plantations in the tropical regions of the world, meets with difficulties resulting from the habits of the insects. Chemical and biological control methods have not yet produced economically worthwhile results. It was therefore proposed, under the Joint Project established by the UN Special Fund and the South Pacific Commission for devising a method of controlling the main pest in this group, *Oryctes rhinoceros*, that the possibility be investigated of using against these coleoptera the technique of releasing males sterilized by radiation. Studies have been made involving the two most important species *O. monoceros* Ol. and *O. rhinoceros* L. The first stage was the development of a continuous breeding method, which had not hitherto been possible because of the large-scale slaughter arising from fungus attacks by *Metarrhizium anisopliae* Metsch. For the larvae the technique used involved first individual rearing, followed by group rearing tests with 100 or so insects in tanks. The average temperature in both cases was close to 30°C and the insects were fed on a mixture of rotten wood and leaf mould mixed with the faeces of domestic animals. The adults, fed on banana slices, are placed in troughs or tanks containing sieved earth. It was thus possible to rear *Oryctes* in several successive cycles, avoiding the epizooic stage of *Metarrhizium*, with a multiplication rate from one generation to the next of close on 10. Tests on radiation sterilization procedures taking as variables the gamma-ray dose, the stage of the insect and the physiological state of the imago, showed that *O. monoceros* adults were sterilized by a single application of 4000 rad, with a slight reduction in their length of life. The same was true of *O. rhinoceros*, which however required a dose greater than 6000 rad to sterilize it. The mating urge and sexual vigour of the irradiated insects are, however, reduced; competitive trials between irradiated and normal males are described and commented on. Apart from the immediate effects of gamma-rays, a possible deferred effect on the progeny of females mated with partially sterilized males was also envisaged.

APPLICATION AUX *Oryctes* (COLEOPTERES: SCARABAEIDAE) DE LA METHODE DU LACHER DE MALES STERILES. La lutte contre les *Oryctes*, qui commettent des dégâts considérables dans les plantations de palmiers et de cocotiers dans les régions tropicales du globe, se heurte à des difficultés dues aux mœurs de ces insectes. Les méthodes chimiques de même que les essais de lutte biologique n'ont pas abouti jusqu'à présent à des résultats économiquement valables. Aussi il a été proposé, dans le cadre du Projet conjoint du Fonds spécial des Nations Unies et de la Commission du Pacifique Sud, chargés de mettre au point la lutte contre le principal ravageur de ce groupe, *O. rhinoceros*, d'étudier les possibilités d'application à ces coléoptères des procédés de lutte autocide par lâchers de mâles stérilisés par irradiation. Les recherches effectuées par l'auteur et son équipe ont porté sur les deux espèces les plus importantes: *O. monoceros* Ol. et *O. rhinoceros* L. Dans une première étape on a cherché à réaliser un élevage permanent qui n'avait jamais pu être obtenu du fait des hécatombes provoquées par la muscardine verte à *Metarrhizium anisopliæ* Metsch. Pour les larves, la technique utilisée a été d'abord l'élevage individuel; ensuite il a été procédé à des essais d'élevage par groupes d'une centaine d'insectes dans des bacs. La température moyenne dans les deux cas était voisine de 30°C et l'alimentation assurée par un mélange

* Recherches effectuées sous contrat (Projet conjoint du Fonds spécial des Nations Unies et de la Commission du Pacifique Sud, «Rhinoceros Beetle Project», Apia, Samoa occidentales).

de bois décomposé ou de terreau de feuilles avec des fèces d'animaux domestiques. Les adultes alimentés avec des rondelles de banane sont mis en ponte dans des seaux ou des bacs renfermant du terreau criblé. L'élevage des Oryctes a pu ainsi être réalisé en plusieurs cycles successifs, sans épizootie à Metarrhizium, avec un taux de multiplication voisin de 10 d'une génération à l'autre. Les essais sur les modalités de stérilisation par irradiation en fonction de la dose de rayons gamma, du stade de l'insecte et de l'état physiologique des imagos ont montré que les adultes de O. monoceros étaient stérilisés par une seule application de 4000 rad avec une faible réduction de la longévité des individus. Les mêmes phénomènes s'observent chez O. rhinoceros, qui demande toutefois une dose supérieure à 6000 rad pour aboutir à la stérilisation. La tendance à l'accouplement, la vigueur génitale des insectes irradiés apparaissent toutefois amoindries; les essais de compétition entre mâles irradiés et mâles normaux sont exposés et commentés. En dehors des effets immédiats des rayons gamma, une éventuelle action différée sur la descendance de femelles accouplées avec des mâles partiellement stérilisés a été recherchée.

1. DIFFICULTES DE LA LUTTE CONTRE LES Oryctes

Les Oryctes constituent un des principaux exemples de ravageurs d'importance économique considérable contre lesquels, en dépit des progrès réalisés depuis 20 ans dans le domaine phytosanitaire, il n'existe pas encore de méthode de lutte rationnelle et efficace, ainsi que nous avons eu l'occasion de le rappeler précédemment [1]. On connaît au moins 25 espèces d'Oryctes nuisibles aux palmiers et aux cocotiers, sans parler des autres genres de Dynastides tels que les Strategus, Scapanes et autres Xylotrupes répandus dans toutes les régions tropicales et équatoriales. Les deux espèces les plus importantes sont O. monoceros d'Afrique et O. rhinoceros d'Asie et d'Océanie qui représentent, surtout la dernière, un véritable fléau dans certaines plantations. D'après Cumber [2], en effet, deux insectes par arbre suffisent pour provoquer des dégâts très importants, et Hinckley [3] pense que, dans les conditions du Pacifique, lorsqu'il y a un insecte pour 19 cocotiers cette culture n'est plus rentable.

Les difficultés de la lutte contre ces insectes sont liées à leur mode de vie et leur comportement. Les larves vivent dans les matières végétales en décomposition, notamment les palmiers morts, tandis que les adultes ne sortent que la nuit pour aller s'attaquer à la base des jeunes palmes, dans lesquelles ils forent une galerie où ils s'abritent pendant plus ou moins longtemps avant de retourner pondre dans les gîtes larvaires. Ceux-ci sont dispersés à la fois dans les plantations et dans la brousse environnante. La destruction de ces gîtes est une des mesures essentielles pour limiter la prolifération des Oryctes; aussi est-elle prescrite par la législation dans beaucoup de pays. Mais elle n'est pas toujours facile à réaliser.

En dehors de procédés empiriques tels que l'extraction des adultes des galeries et la destruction des larves dans les gîtes naturels ou dans des bûches pièges disposées à cet effet, la méthode de lutte la plus couramment employée est le traitement des couronnes par un mélange d'insecticide et de sciure de bois placé à l'aisselle des plus jeunes feuilles. Mais il est nécessaire de répéter cette opération très souvent (deux ou trois fois par mois en Côte-d'Ivoire, par exemple) [4], de sorte que le prix de revient est élevé, de l'ordre de 4000 ou 5000 F CFA/ha·an, pour une efficacité douteuse.

De multiples essais de lutte biologique ont été entrepris depuis le début du siècle pour obtenir un contrôle naturel de ces ravageurs, soit par l'introduction d'insectes parasites, tels que diverses espèces de

Scolia (Hyménoptères Scoliidae), ou prédateurs, tels que des Coléoptères Elatérides ou Scarabides et des Hémiptères Réduvides, soit par traitement de certains gîtes larvaires par le champignon Metarrhizium anisopliae.

Dans le premier cas, l'acclimatation de certains entomophages a été obtenue à plusieurs reprises dans différentes îles du Pacifique (Fidji, Palau, Samoa), mais jusqu'à présent aucun de ces insectes n'a permis de réduire les dégâts d'Oryctes. Dans le deuxième cas, quelques résultats encourageants ont été enregistrés avec le champignon, mais il n'a pas été possible de les généraliser en dépit des très nombreuses tentatives d'utilisation de ce pathogène effectuées dans diverses régions de l'aire géographique de O. rhinoceros et malgré la fréquence des épizooties naturelles dues à cette muscardine.

2. POSSIBILITES OFFERTES PAR LA LUTTE AUTOCIDE

Devant ces échecs des méthodes chimiques et biologiques et du fait du comportement de ces insectes, dont le stade imaginal est le seul vraiment accessible, au moins dans certaines périodes, nous avons proposé d'examiner les possibilités d'application aux Oryctes et notamment à O. rhinoceros des méthodes de lutte autocide qui ont fait leur preuve pour d'autres ravageurs, depuis les succès rencontrés aux Etats-Unis dans la lutte contre la mouche du bétail, Cochliomyia hominivorax.

D'autre part, l'expérimentation de Horber [5, 6] sur le hanneton commun, espèce de la même famille, constituait un précédent favorable pour le recours aux lâchers de mâles stériles en vue de tenter une éradication de O. rhinoceros.

Cet objectif a été pris en considération par le Conseil de direction du Projet conjoint du Fonds spécial des Nations Unies et de la Commission du Pacifique Sud, établi depuis 1964 en vue de mettre au point la lutte contre O. rhinoceros et les insectes apparentés. Aussi l'Institut de recherches pour les huiles et oléagineux (IRHO) et la Station de recherches de lutte biologique et de biocœnotique de La Minière (INRA) bénéficient-ils d'un contrat de recherche dans le cadre de ce Projet, en vue de préciser les possibilités d'application aux Dynastides de la lutte autocide par irradiation.

Dans les cas des Oryctes, comme pour les autres insectes, ainsi que Féron [7] l'a souligné précédemment, l'emploi de cette méthode de lutte suppose des recherches préalables dans trois principales directions: la mise au point d'un élevage permanent, la comparaison du comportement des insectes stérilisés par rapport à celui des insectes normaux, l'étude en plantation de l'éthologie et de l'écologie pour parvenir à une méthode d'estimation des populations.

Nous nous sommes attachés depuis deux ans à la réalisation des deux premières conditions : mise au point de l'élevage et examen en laboratoire des effets de l'irradiation, les recherches écologiques étant conduites parallèlement, soit dans le Pacifique Sud par les soins des chercheurs du Projet ou de l'Office de la recherche scientifique et technique d'outre-mer (ORSTOM) dans le cas de O. rhinoceros, soit en Côte-d'Ivoire par l'IRHO dans le cas de O. monoceros.

3. REALISATION D'UN ELEVAGE PERMANENT D'Oryctes

La méthode d'élevage de ces Dynastides tropicaux a été adaptée à partir des résultats de l'expérimentation antérieure [8] que nous avions conduite sur deux espèces européennes de la même famille, Oryctes nasicornis L. et Phyllognathus silenus F. Celle-ci avait mis en évidence qu'en dehors d'une température suffisante (25 à 30°C) le facteur essentiel pour éviter les hécatombes dues à M. anisopliae était l'alimentation des larves avec un mélange de matière végétale en décomposition (terreau ou bois pourri) et d'excréments d'origine animale. La ponte des adultes issus des larves élevées dans ces conditions était obtenue sans difficulté dans un seau de 10 litres en polyéthylène rempli aux deux tiers de terreau criblé et fermé par une feuille de polyane pour conserver l'humidité du milieu, car ces insectes ne s'alimentent pas à l'état imaginal.

Pour les Oryctes tropicaux, dont les adultes se nourrissent, il a été nécessaire de procéder à un certain nombre d'essais portant notamment sur l'alimentation et la nature du récipient d'élevage pour les larves et pour les imagos, ainsi que sur l'influence du groupement des individus, avant de parvenir à une méthode éprouvée. Ces essais ont porté essentiellement sur O. monoceros à partir d'un millier d'adultes et de larves expédiés en 13 envois successifs de la plantation de l'IRHO à Port Bouet (Côte-d'Ivoire) de mai 1964 à septembre 1965, et sur O. rhinoceros à partir de plus de 4000 œufs reçus d'Apia (Samoa occidentales) de mars à novembre 1966.

Deux publications [9] ont fait le point des résultats acquis dans l'obtention en laboratoire, en toute saison, de ces deux espèces, et présenté les principales caractéristiques de l'évolution de ces insectes. La poursuite de l'expérimentation nous a permis d'apporter depuis certaines améliorations, en particulier dans la fécondité des femelles, de sorte que l'ensemble des données actuelles en ce domaine peut se résumer de la façon suivante.

3.1. Elevage des larves

Il peut être effectué, soit en boîtes individuelles de 500 ml de capacité, soit dans des récipients en polyéthylène de 35 litres contenant 20 à 30 larves, soit en bacs de 600 litres renfermant une centaine d'individus. La précaution essentielle est d'utiliser le mélange de matières en décomposition et de fèces en respectant les exigences de température et d'humidité (30°C et 65% HR). De multiples essais comparatifs de la valeur de différents substrats (terreau, bois décomposé, sciure) et des excréments des principaux animaux domestiques (cheval, vache, porc, mouton) ont montré que la combinaison bois de feuillu à fibre courte (hêtre, châtaignier, orme) + bouse de vache constituait le meilleur milieu pour le développement larvaire, en particulier pour limiter la mortalité par mycose, ainsi qu'en témoigne le tableau I récapitulant 45 essais portant chacun sur 50 à 200 individus.

Les élevages de groupes de O. rhinoceros donnent des résultats très hétérogènes et variables d'un lot à l'autre à cause de la plus grande durée du cycle évolutif de cette espèce, qui demande 6 à 7 mois de l'éclosion de l'œuf à la formation de l'imago, alors que pour O. monoceros il suffit de

TABLEAU I. DEVELOPPEMENT DES Oryctes SUIVANT LE MILIEU D'ELEVAGE

Espèce	Elevage	Substrat	Larves L_1 en élevage	Imagos (Ip) formés	Ip/100 L_1	Metarhizium (%)
O. monoceros	Individuel	bois + bouse	874	604	70	1,6
	En groupe	sciure + bouse	784	329	42	19
	En groupe	bois + bouse	328	243	74	1
O. rhinoceros	Individuel	terreau + bouse	735	271	36	25
		bois + bouse	989	526	53	6
	En groupe	terreau + bouse	252	30	12	60
		sciure + bouse	200	60	30	20
		bois + bouse	512	164	32	20

Ip = Insectes parfaits.

$3\frac{1}{2}$ mois à 4 mois. Les 3 mois supplémentaires nécessaires pour O. rhinoceros sont les plus difficiles pour le maintien de la structure et de l'humidité du milieu, qui tend à se dessécher progressivement. Dans ce cas, de même qu'après un transfert des larves dans un milieu d'humidité brusquement trop élevée, la mycose se manifeste et détruit la quasi-totalité de l'élevage considéré, d'où, pour cette espèce, des rendements très différents d'un bac à l'autre pour un même substrat. Ces difficultés sont en cours de solution, en particulier par le recours à des enceintes de volume plus restreint pour la fin de la vie larvaire et la nymphose. En utilisant le bois et la bouse de vache on obtient par ce procédé un rendement de 70% d'adultes formés par rapport au nombre de larves nouvelles nées mises en élevage, de la même façon que pour O.monoceros.

Nous nous proposons d'analyser les facteurs physiques ou chimiques des substrats expérimentés déterminant le meilleur développement des larves et la limitation de la mycose. Il semble que les deux constituants interviennent, car des tests préliminaires ont montré que le bois décomposé seul ou le terreau seul ne permettait pas l'élevage de ces insectes. D'autre part, 11 essais effectués avec O. monoceros alimenté en élevage individuel avec des fèces de différents animaux domestiques (vache, cheval, porc, mouton) mélangées, soit au bois décomposé, soit au terreau, ont mis en évidence la supériorité du bois, notamment à l'égard de M. anisopliae (tableau II).

TABLEAU II. INFLUENCE DE LA NATURE DU SUBSTRAT SUR LE DEVELOPPEMENT LARVAIRE DE O. monoceros

Substrat	L_1 en élevage	Ip formés	Rendement %	Metarrhizium (%)
Bois + fèces	270	218	80	3
Terreau + fèces	311	136	43	36

3.2. Elevage des adultes

Dans l'état actuel de nos expériences sur l'influence de diverses cages et de différents substrats, il apparaît que le terreau de couche de deux ans représente le milieu le plus favorable au dépôt des œufs des deux espèces, dont l'alimentation est assurée par des rondelles de banane disposées en surface et renouvelées deux fois par semaine. Les femelles sont aptes à la reproduction dans les jours qui suivent leur sortie de la dépouille nymphale, de sorte que pour ces deux Dynastides la ponte peut commencer au bout de 2 semaines. Chez O. monoceros, elle peut se prolonger pendant 4 mois, chez O. rhinoceros pendant 6 mois, délais qui correspondent approximativement à la longévité des femelles. Dans les dispositifs expérimentés, la fécondité de l'espèce africaine est plus de deux fois moindre que celle de l'insecte asiatique (30 œufs en moyenne par femelle contre 74 dans les meilleures conditions), avec des pontes pouvant atteindre 70 œufs pour certaines femelles de O. monoceros et 150 à 180 œufs pour O. rhinoceros. Le groupement des individus dans des récipients d'un certain volume paraît favoriser la survie et la ponte des femelles, surtout chez O. monoceros, comme l'indique le tableau III. Pour O. rhinoceros, les différences entre les divers types d'enceintes de ponte sont beaucoup moins nettes. Il en est de même pour la fertilité des œufs exprimée par le pourcentage de larves écloses à partir des pontes obtenues.

Dans la pratique, le bac de 50 litres avec 10 couples est le procédé le plus commode pour obtenir la ponte des Oryctes, s'il n'est pas nécessaire de faire des études à l'échelon individuel.

4. ETUDE DE LA REPRODUCTION DES Oryctes

La mise au point d'un élevage de ces Dynastides nous permet de disposer d'insectes d'âge et d'état physiologique connus et, ainsi, d'entreprendre l'examen des principaux caractères de la fécondation et de la reproduction des insectes normaux, de façon à pouvoir interpréter les phénomènes produits par l'irradiation.

Les expériences en cours ont pour but de déterminer le rythme d'évolution des ovocytes à 30°C et l'influence de l'âge des individus des

deux sexes sur les délais et le nombre d'accouplements. La dissection de 4 femelles tous les 2 ou 5 jours indique l'état du tissu adipeux, le nombre et la taille des ovocytes et la présence ou non de spermatophores dans la poche copulatrice. En outre, les femelles de même origine sont élevées jusqu'à la ponte pour évaluer la fécondité des insectes considérés. Il a été procédé à l'étude de couples constitués, soit d'insectes formés depuis quelques jours, soit d'animaux vierges alimentés pendant 1 mois avant la mise en élevage et maintenus ensemble pendant des délais variables de 2 semaines à 2 mois. D'autre part, les résultats enregistrés avec des couples isolés ont été comparés à ceux obtenus dans les bacs renfermant un groupe de 10 couples.

Il apparaît que la majorité des accouplements s'effectue 8 à 12 jours après la mise en présence des deux sexes, au moment où le tissu adipeux des femelles commence à être beaucoup moins abondant et à prendre l'aspect de ballonnets pleins d'air, parallèlement à une ovogenèse active se manifestant par l'existence de 3 à 5 ovocytes dont le plus gros et le plus ancien est en début de vitellogenèse. Le nombre de mâles et de femelles dans la même enceinte ne paraît pas intervenir.

Un essai portant sur l'élevage de O. rhinoceros des deux sexes d'âges différents (mâles de quelques jours avec des femelles maintenues 6 semaines à jeun à 20°C et vice-versa) a montré qu'il n'y a pas de différence de fécondité (40 œufs par femelle en moyenne) ni de fertilité (85%), ni de délais pour la première ponte (2 semaines à 30°C) entre les deux lots. Par contre, les mâles âgés de 6 semaines sont susceptibles de féconder les femelles dès les premiers jours alors que pour des mâles jeunes les premiers accouplements ne s'observent qu'à partir du 10e jour, avec possibilité de deux coïts pour le même couple dans les 15 jours qui suivent la mise en élevage. En outre l'ovogenèse est plus rapide et plus homogène dans le cas des femelles jeunes.

A la différence de M. melolontha, les femelles d'Oryctes sont donc aptes à la reproduction dans les jours qui suivent la sortie de la nymphe, tandis que pour les mâles une période de maturation d'au moins 1 semaine est nécessaire pour permettre l'accouplement. Ces observations éthologiques devront naturellement être confirmées et précisées par une étude histologique.

L'accouplement n'est pas indispensable à l'évolution des ovocytes : 10 femelles vierges de O. rhinoceros en élevage groupé à 30°C ont déposé un total de 165 œufs en 2 à 6 semaines. Mais la fécondité (16 œufs par femelle) et la longévité (inférieure à 2 mois) de ces insectes sont inférieures à celles de femelles accouplées, ce qui amène à penser que des phénomènes analogues à ceux mis en évidence par Landa [10] chez le hanneton commun se produisent également chez les Dynastides : utilisation des restes de spermatophores comme source complémentaire de protéines pour l'élaboration du vitellus.

Cette hypothèse est confirmée par la différence de fécondité enregistrée dans un essai préliminaire sur le pouvoir fécondant du mâle de O. rhinoceros. Dans trois lots de 12 femelles élevées avec 1, 3 et 12 mâles, la fécondité moyenne par femelle a été, respectivement, de 35, 30 et 63 œufs.

D'après les nombreuses observations que nous avons eu l'occasion de faire dans nos études sur la ponte, les spermatozoïdes conservent leur pouvoir fécondant dans les voies génitales femelles pendant 2 à 3 mois chez O. monoceros et jusqu'à 5 mois chez O. rhinoceros, à en

TABLEAU III. INFLUENCE DES CONDITIONS DE PONTE SUR LA FECONDITE ET LA FERTILITE DES Oryctes à 30°C

Espèce	Dispositif	Nombre de couples par enceinte	Nombre total de femelles	Nombre d'œufs	Fécondité moyenne par femelle (nombre d'œufs)	Fertilité moyenne des œufs (%)
O. monoceros	seau de 10 litres	1	85	1150	13,5	26
	seau de 10 litres	3	33	512	15,5	64
	bac de 50 litres	10	113	3331	30	78
O. rhinoceros	bac de 5 litres	1	14	1014	72	72
	seau de 10 litres	1	7	390	56	70
	seau de 10 litres	3	15	419	28	80
	bac de 35 litres	5	14	830	60	75
	bac de 50 litres	10	37	2611	74	80

juger par la fertilité des œufs déposés dans les élevages de couples dont le mâle est mort prématurément.

5. STERILISATION DES MALES PAR IRRADIATION

5.1. Définition des doses stérilisantes

L'irradiation a été réalisée par un irradiateur chargé de ^{60}Co dont l'activité totale était de 1500 Ci, ayant un débit de dose au moment de nos essais de 1100 rad/min. En fonction des données de Horber [5, 6] pour M. melolontha une dose de 9000 rad a été utilisée, en première approximation de la sensibilité de ce genre d'insectes, pour des mâles de O. monoceros, dont nous avons eu l'élevage avant celui de O. rhinoceros. Pour l'irradiation, les insectes étaient placés par groupes de trois dans une boîte en polystyrène de 8 cm de haut sur 7,5 cm de diamètre remplie de tourbe humidifiée. Ils étaient ensuite mis en dispositif de ponte à 30°C avec des femelles normales, soit par couples, soit par groupes de 10 couples. Des adultes de même âge et de même origine, non irradiés, servaient de témoins pour chaque dose. Dans une première étape nous avons cherché à déterminer la dose minimale de rayons gamma stérilisant les mâles et réduisant le moins possible leur longévité, en fonction de l'âge des insectes : imagos formés depuis quelques jours, et imagos conservés à jeun à 20°C pendant 3 semaines. Le tableau IV récapitule les résultats concernant O. monoceros et le tableau V les données relatives à O. rhinoceros.

Ces tableaux font ressortir la sensibilité moindre de O. rhinoceros à l'irradiation. Alors que 4000 rad assurent la stérilisation de O. monoceros et que 3000 rad seraient suffisants pour cette espèce, il faut au moins 6000 rad pour O. rhinoceros, dans l'état actuel de nos recherches. Des essais sont en cours en utilisant des doses supérieures, allant jusqu'à 10 000 rad, pour fixer la dose optimale. Dans les deux cas l'âge des adultes ne paraît pas avoir d'influence marquée sur la sensibilité aux rayons gamma et la longévité des mâles traités de O. monoceros est peu affectée par les doses stérilisantes, tandis que 6000 rad réduisent les délais de survie des adultes de O. rhinoceros par rapport aux insectes normaux.

Dans trois lots de 10 couples de O. rhinoceros, les deux sexes ont été irradiés simultanément à raison de 3000 rad, 4000 rad et 5000 rad. Seules les femelles soumises à 3000 rad ont déposé des œufs, en nombre très restreint (23 au total pour 10 femelles) et aucune éclosion n'a été enregistrée. La longévité des femelles traitées a été analogue à celle des mâles : 6 semaines environ. Des expériences complémentaires permettront de préciser ces premières données sur la sensibilité des deux sexes aux rayons gamma.

De premiers essais d'irradiation aux rayons X ont été effectués parallèlement pour O. rhinoceros. Des séries de 5 imagos ont été placées dans des boîtes en polystyrène de 10 cm × 7 cm, au contact du générateur sous une tension de 200 kV, à 12,5 mA, à un débit de dose de 111 rad/min, en interposant un filtre en cuivre de 0,5 mm. Deux doses ont été utilisées pour des lots de 10 mâles : 3000 rad et 5000 rad. La fécondité des femelles et la fertilité des œufs (67% d'œufs fertiles pour 3000 rad et 48% pour 5000 rad) sont du même ordre de grandeur que pour les mêmes doses

TABLEAU IV. ACTION DES RAYONS GAMMA SUR O. monoceros

Age du mâle	Dose (rad)	Nombre de mâles	Longévité moyenne du mâle (semaines)	Fécondité moyenne par femelle (nombre d'œufs)	Fertilité moyenne des œufs (%)
3 semaines	0	7	8	16	50
3 semaines	9000	17	6	10	3
3 jours	9000	16	7	20	3
3 jours	Témoin	6	8	17	80
3 semaines	Témoin	12	8	40	72
3 jours	6000	30	6	12	0
3 semaines	6000	25	5	12	0
3 jours	Témoin	10	10	24	62
3 semaines	Témoin	15	7	10	59
3 jours	4000	11	8	30	2
3 semaines	4000	20	7	24	0
3 jours	3000	10	5	35	5
3 semaines	3000	10	8	51	4
3 jours	2000	10	6	36	12

TABLEAU V. ACTION DES RAYONS GAMMA SUR O. rhinoceros

Age du mâle	Dose (rad)	Nombre de mâles	Longévité moyenne du mâle (semaines)	Fécondité moyenne par femelle (nombre d'œufs)	Fertilité moyenne des œufs (%)
3 jours	Témoin	12	10	74	86
3 semaines	Témoin	10	9	75	72
3 jours	3000	10	9	95	76
3 semaines	3000	10	5	15	27
3 jours	4000	20	9	55	62
3 semaines	4000	20	6	56	66
3 jours	5000	20	9	55	17
3 semaines	5000	25	7	48	5
1 semaine	6000	10	6	27	2

TABLEAU VI. COMPETITIVITE DES MALES IRRADIES PAR RAPPORT AUX MALES NORMAUX
(Ponte à 30°C par lots de 9 ou 10 couples)

Espèce	Dose (rad)	Mâles irradiés / normaux	Nombre total de couples	Fécondité moyenne par femelle (nombre d'œufs)	Fertilité moyenne des œufs (%)
O. monoceros	4000	2	18	49	62
		4	30	47	64
	Témoin	-	10	22	73
O. rhinoceros					
	5000	2	36	29	60
		4	10	62	81
	6000	2	36	61	76
		4	10	52	82
	Témoin	-	10	52	80

de rayons gamma. Mais nous poursuivons la comparaison des deux modes d'irradiation afin de vérifier que la nature du rayonnement n'intervient pas, ainsi que Amy [11] l'a démontré sur les œufs de Habrobracon.

5.2. Compétition entre mâles normaux et mâles irradiés

Un des points importants à établir pour le calcul théorique du nombre d'insectes stériles à lâcher dans la population considérée est la vigueur génitale et la compétitivité sexuelle des mâles irradiés. Dans ce but, des lots de 10 couples de O. monoceros ont été constitués en associant 10 femelles normales avec 8 (ou 6) mâles irradiés à 4000 rad et 2 (ou 4) mâles non traités. Dans le cas de O. rhinoceros nous avons opéré, soit avec 9 couples (6 mâles irradiés et 3 mâles normaux), soit avec 10 couples (8 mâles irradiés et 2 mâles normaux), dont les insectes traités avaient été soumis pour partie à 5000 rad et pour partie à 6000 rad. Le tableau VI montre qu'il est nécessaire d'augmenter la proportion de mâles stériles pour agir sur la fertilité des œufs, ce qui est en accord avec les données de la littérature : Proverbs et Newton [12] indiquent qu'il faut 20 mâles stériles de carpocapse par mâle normal pour obtenir une réduction rapide des populations en verger, et la même proportion a été trouvée par Howland et al. [13] pour Trichoplusia ni.

5.3. Effets de l'irradiation sur la descendance

En plus de l'influence directe des rayons gamma sur la fécondité et la fertilité des adultes, nous avons abordé l'étude des effets différés éventuels sur la génération issue des œufs déposés par des femelles accouplées avec des mâles irradiés. Plusieurs centaines de larves provenant d'adultes de O. rhinoceros soumis à 3000, 4000 ou 5000 rad sont élevées individuellement. En raison des délais de développement et des différences individuelles ces élevages ne sont pas terminés. Cependant, un grand nombre d'adultes sont maintenant formés et en cours de ponte. Dans l'ensemble il ne paraît pas y avoir de différence marquée dans la durée de l'évolution des larves, le poids des imagos formés et la fécondité de ceux-ci par rapport aux insectes issus des élevages normaux.

CONCLUSIONS

Un certain nombre de données positives ont pu être acquises ces deux dernières années au cours de notre expérimentation sur les modalités d'application aux Oryctes des méthodes de lutte autocide par irradiation. Un des points fondamentaux, non seulement pour ces recherches mais aussi pour toute étude physiologique ou pathologique précise de ces Dynastides, est la mise au point d'une technique d'élevage en laboratoire assurant la disponibilité en permanence d'insectes d'âge bien déterminé. C'est grâce à ces élevages, dans lesquels la mycose à Metarrhizium est contrôlée, que nous avons pu entreprendre l'examen de la biologie de la reproduction des deux espèces les plus importantes au point de vue économique, ainsi que la définition des normes de stérilisation et des processus de compétition entre individus irradiés et insectes normaux.

Les résultats résumés dans ce mémoire ont encore un caractère préliminaire du fait de la durée du cycle évolutif des espèces considérées et de la nécessité de procéder à des répétitions en nombre suffisant pour permettre des conclusions définitives. La poursuite de nos recherches a pour objet d'aboutir à de telles conclusions notamment en ce qui concerne:
-les différentes étapes de la vie sexuelle des mâles et des femelles normaux et irradiés, par une étude histologique appropriée,
-la comparaison de l'effet stérilisant des diverses sources de radiations (X et gamma) avec différents débits,
-la délimitation de la proportion d'individus stérilisés nécessaires pour provoquer une réduction suffisante du taux de prolifération de la population.

Mais, en dehors de ces études de laboratoire, l'application de cette méthode de lutte aux Oryctes suppose l'amélioration de nos connaissances sur le comportement des adultes et sur la dynamique des populations en milieu naturel, et surtout la mise au point d'une méthode de capture par piégeage des imagos.

Cette méthode est nécessaire à la fois pour suivre les niveaux de population et pour récolter en grand nombre les insectes qui seront soumis à l'irradiation avant d'être relâchés. Dans le cas des Oryctes, en effet, il nous paraît très difficile d'envisager la stérilisation d'insectes provenant d'élevages pour deux raisons : les élevages de masse demanderaient des moyens considérables, en particulier à cause de la longueur du cycle évolutif de ces Coléoptères qui n'a rien de commun avec celui des Diptères. D'autre part, les dégâts sont occasionnés par les adultes dont il y a lieu, par conséquent, d'accroître le moins possible le nombre, même de façon temporaire.

Ce sont de telles études, qui sont activement conduites par les spécialistes du projet «Rhinoceros Beetle» à Apia, notamment par la recherche d'attractifs chimiques, qui doivent permettre d'aboutir à l'expérimentation sur le terrain de procédés de lutte autocide, méthode qui nous paraît être une des possibilités offertes par l'entomologie moderne pour faire face au grave problème des Oryctes.

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REFERENCES

[1] HURPIN, B., Résultats et perspectives de la lutte biologique contre les Oryctes, Oléagineux 21 2 (1966) 77-82.

[2] CUMBER, R. A., Le rhinocéros du cocotier aux Samoa Occidentales, Commission du Pacifique Sud, Doc. Techn. n° 107 (1957) 1-76 (Miméo).

[3] HINCKLEY, A. D., Les dégâts causés par l'Oryctes rhinoceros (L.) dans les cocoteraies des îles du Pacifique, Bull. Pacif. Sud. 16 4 (1966) 43-44.

[4] MARIAU, D., Lutte chimique contre l'Oryctes, Résultats préliminaires, Oléagineux 22 3 (1967) 155-158.

[5] HORBER, E., "Eradication of white grub (Melolontha vulgaris F.) by the sterile-male technique", Radiation and Radioisotopes Applied to Insects of Agricultural Importance" (Proc. Symp. Athens, 1963), IAEA, Vienna (1963) 313-32.

[6] HORBER, E., Maikäfer gegen Maikäfer: Ein Versuch zur Tilgung eines Engerlingsherdes des Feldmaikäfers (Melolontha vulgaris Fabr.) mit röntgenbestrahlten Männchen, Waldhygiene 6 6 (1966) 161-70.

[7] FERON, M., La lutte contre les insectes par les méthodes autocides, Revue Zool. agric. appl. 26 (1963) 37-48.

[8] HURPIN, B., FRESNEAU, M., Elevage de deux dynastides, Oryctes nasicornis L. et Phyllognathus silenus F. (Coléopt. Scarabæidæ), Revue Path. vég. Ent. agric. Fr. 43 2 (1964) 75-96.

[9] HURPIN, B., MARIAU, D., Contribution à la lutte contre les Oryctes nuisibles aux palmiers, Mise au point d'un élevage permanent en laboratoire, C. r. hebd. Séanc. Acad. Agric. Fr. (1966) 178-186.
HURPIN, B., FRESNEAU, M., Contribution à la lutte contre les Oryctes nuisibles aux palmiers, 2. Elevage en laboratoire de O. rhinoceros L., Oléagineux 22 11 (1967).

[10] LANDA, V., Origin, development and function of the spermatophore in cockchafer (Melolontha melolontha L.), Acta Soc. Ent. Čechoslov. 57 (1960) 297-316.

[11] AMY, R.L., A comparative study of the effect of β-rays, γ-rays and X-rays on development in Habrobracon, Radiat. Res. 3 (1955) 166-81.

[12] PROVERBS, M.O., NEWTON, J.R., Suppression of the reproductive potential of the codling moth by gamma irradiated males in caged orchard trees, J. econ. Ent. 55 (1962) 934-36.

[13] HOWLAND, A.F., VAIL, P., HENNEBERRY, T.J., Results of cage experiments with sterile male releases and a chemosterilant technique for control of cabbage looper populations, J. econ. Ent. 59 1 (1966) 194-96.

DISCUSSION

G. LE MASNE: Do you have any information on behavioural aspects influencing competition between irradiated and non-irradiated males?

B. HURPIN: No, I'm afraid not.

EFFECT OF RELEASING STERILE *Scirpophaga nivella* F. ON CROP DAMAGE AND INSECT POPULATION DENSITY IN SUGAR CANE PLANTATIONS

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Abstract

EFFECT OF RELEASING STERILE *Scirpophaga nivella* F. ON CROP DAMAGE AND INSECT POPULATION DENSITY IN SUGAR CANE PLANTATIONS. Successful use of the sterile-male technique to control insect populations was reported in several countries. Certain characteristics of a candidate insect must be considered before employment of this technique. Since 1965, experiments have been performed at the State Sugar Academy in Jogjakarta, Indonesia, to examine these characteristics in the white top borer (*Scirpophaga nivella* F.), an important sugar cane pest, and to determine the suitability of the sterile-male method. Several important characteristics were found suitable, i.e. copulation ability did not decrease after radiation, and rearing on an artificial medium is possible for all life cycle stages. Radiation at the pupal stage induced sterility in the adult. Preliminary experiments in a sugar cane plantation were performed to determine the effect of sterile insect release on the attack intensity and population density. The number of released sterile insects approximately equalled that of the natural population. Release was carried out at a time when cane was susceptible to attack in an area which had previously reported 10% annual crop loss due to this insect's activity.

Released sterile insects caused a decrease in the attack intensity and the population density. An examination carried out just before harvesting, after release of the insects had been terminated, revealed that the insect population density at the control plots was roughly equal to that at the release plots. This indicated that the release of the sterile insects would be effective in suppressing the increase of the population if it were constantly done. Another fact that supports this opinion is that although the figures shown by the control plots and release plots just before harvesting were almost equal, the damage in the release plots was only to the leaves. Thus, if we considered that the damage caused by this pest on sugar cane 10 months old or older may be ignored, control could be accomplished by releasing sterile insects during the sensitive period of sugar cane growth. If however, this pest is to be completely eradicated, the release of the sterile insects must be done constantly during the growing period of the sugar cane.

1. INTRODUCTION

The white top borer is the most destructive native sugar cane pest in Indonesia. According to research carried out in Indonesia in 1957 at 16 sugar factories, the average loss was 8.9%. If the total sugar production in Indonesia was 1 000 000 tons, then 89 000 tons of sugar were destroyed by the white top borer. The value of the loss, calculated according to the current sugar price in Indonesia, was almost two billion rupiahs [8]. The top borer, in combination with the stem borer, caused 12% damage to the sugar crop. Other countries are concentrating their attention on the stem borer, which, for instance, causes an annual loss of $6 000 000 in the United States of America [1].

The increasing sugar loss in Indonesia is partly caused by a planting system which favours the existence of the white top borer and partly by outmoded control methods. The present control method is the so called 'rogesan' method, i.e. cutting the top of the infested sugar cane.

Control by insecticide was not satisfactory because the greater part of the insect's life is spent within the stem, beyond the reach of insecticides.

The success of the sterile-male technique in controlling different pests in various countries prompted the following experiments to determine whether this technique could be used to control the white top borer in sugar cane. These insects can easily be reared by an artificial method out of their natural condition in the field. In 1965, radiobiological studies indicated that the copulation ability was not reduced.

Pupae were irradiated with a ^{60}Co source owned by the Faculty of Mathematics and Physics, Gadjah Mada University. A very small dose caused the moths to become sterile [5, 6].

This paper describes preliminary experiments on the attack intensity and population density after a release of sterile Scirpophaga nivella F. Further research suggested in the XVIth Dies Natalis speech of the State Sugar Academy [8] is also described.

2. MATERIALS AND METHODS

The following experiments were performed to compare the 'rogesan' control method with the sterile insect release.

2.1. Orientation of sugar cane plantations

Six isolated fields with canes about two months old which had never been treated for any pest were used. These fields were similar with regard to the variety of canes, the kinds of seedling, the severity of pest infestation, the intensity of the eradication attempts, the area involved, etc. Three plots were used for the release of sterile insects and the others were used as control plots.

2.2. Population density calculation and the amount of damage caused by borer attack

For every plot of ground the number of rows and the total number of furrows were determined. Furrows were randomly selected and the per cent of infested stems was ascertained.

Larvae and pupae were removed from the infested stems. Based on the number of larvae and pupae found in the sample furrows the density of insects was determined and the developmental stages were recorded. At the same time both the density of insects and the severity of damage throughout the sample plots were recorded monthly. Every time new furrows were chosen, we used the same number and length of furrows as in the first observation.

2.3. Materials preparation and field research supplies

Pupae as release material were obtained from the Madukismo sugar factory in cane tops. The release containers consisted of flat earthen discs fixed on top of bamboo poles planted in the fields. These bamboo poles were 1½ metres long and each had two discs fixed to the top. The upper disc had a cone at the bottom. Banana leaves were here spread out to hold the pupae, which were then covered with small pieces of banana leaves. The upper disc was put on top of the lower disc, which had a flat bottom and was larger in size than the upper. Water was then poured on the lower container to prevent ants from harming the pupae and to keep the upper container humid. The bottom of the second container was fixed to the top of the bamboo pole.

2.4. Larvae rearing and sterility induction

Larvae collected in the field were laboratory reared on sugar cane until they became pupae. These and all field-collected pupae were exposed to a ^{60}Co source. This radiation was given the morning right before release.

2.5. Sterile insect release

Sterile pupae were released one or two days before eclosion, the releases being carried out before the rainy season. Pupa-release is the best method, since the best mating time for these moths is just after their metamorphosis. This release was done at definite times, the number of insects to be released being made equal to the population density or equalized with the number of female moths estimated to emerge. On three neighbouring release plots 10 bamboo release pillars were planted, and the number of pupa put there each time for release depended upon time, frequency and density (Appendix I).

2.6. Observation

The method of observation has already been outlined above. Both control and release plots were kept under observation, as were the egg colonies and the hatching rate, which have so far yielded no definitive data. Monthly observations were carried out during release time, when the cane was most susceptible to white top borer attack, while final observations were made before harvest time to determine whether the sterile insect release had had any effect.

3. RESULTS

The tables and figures illustrate the results of the examinations.

At the sterile insect release plots (Fig. 1), a decrease of attack intensity and population density was noticed, while at the control plots (Fig. 2) an increase of both occurred.

The first pupal release took place on 13 September 1966, in plots I, II, III which had a fixed area comparison. The following numbers of pupae

were released in succession: 75, 75 and 150, respectively; simultaneously the following damage was observed: 9.40%, 5.16% and 6.28% with population densities of 8.50, 4.45 and 5.71 (Table I). Not all pupae released became moths; the following numbers of moths were observed: 51, 60 and 136. At the second release, on 4 October 1966, the damage to plots I, II and III decreased to 4.35%, 1.54% and 3.27%, respectively,

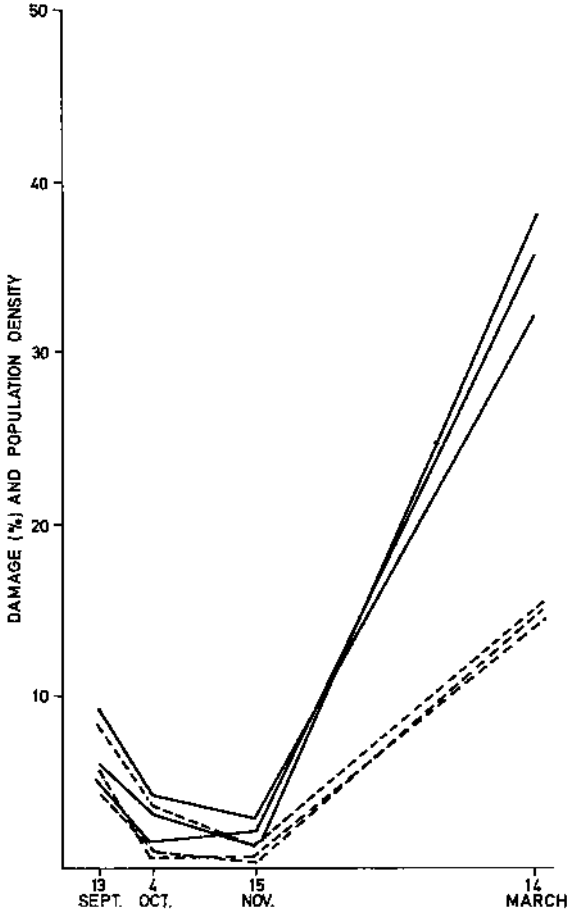

FIG. 1. Damage and population density at release plots.
———: Damage (%)
--------: Population density.

while population densities became 3.79, 1.00 and 0.72. By that time another pupal release had been performal: 54, 54 and 72, respectively, of which 41, 43 and 66 became moths. The third release, on 15 November 1966, produced the following damage in plots I, II and III: 2.85%, 2.04% and 1.25%, while the population density figures were 1.24, 0.25 and 0.37. Of the third pupal release, of 45, 45 and 60 pupae, 44, 45 and

60 became moths. Both observation and insect release were then terminated, because by that time the sugar cane was no longer susceptible to white top borer attack.

The figures on damage and insect population density given below were obtained from the control plots (Table II). The first observation on 7 September 1966 showed the following damage percentages: plots I, II

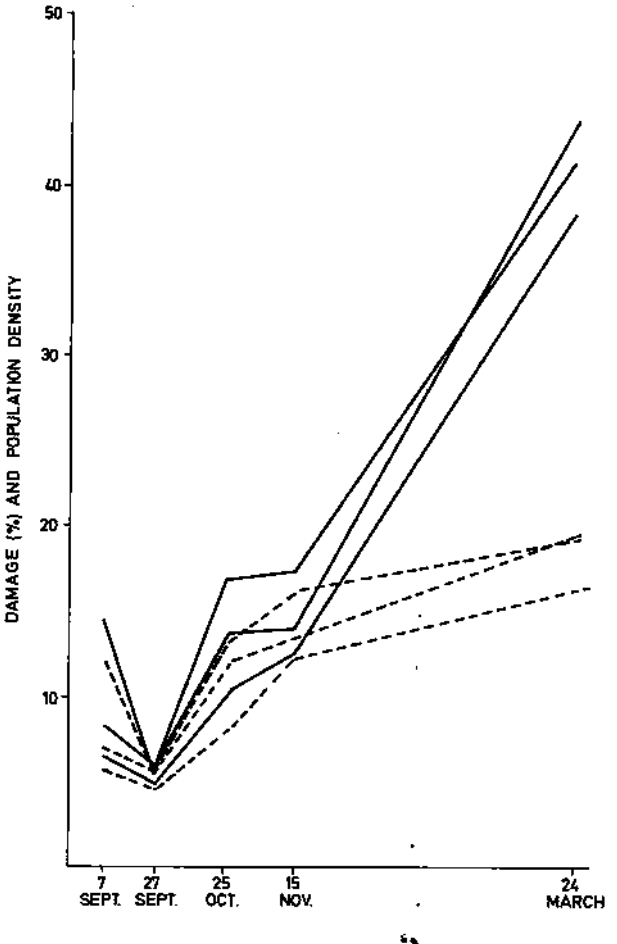

FIG. 2. Damage and population density at control plots.
———: Damage (%)
-------: Population density.

and III, 14.69%, 6.74% and 8.54%, respectively, while the density was 12.50, 5.76 and 7.13. The second observation took place on 27 September 1966, and yielded the following damage figures: 5.76%, 4.79% and 5.87% with population densities of 5.65, 4.50 and 5.46, respectively. Figures for both damage and population density became smaller. On the other hand, a striking increase in these figures was observed in the following

TABLE I. DAMAGE AND INSECT POPULATION DENSITY AT RELEASE PLOTS
(Preliminary experiment on cane fields [7])

Date	Plot No.	Total of pupae		Total stem in furrows		Damage (%)	Total (In sample furrows)		Density
		Release	Remainder	Total number	Infested stem		Larvae	Pupae	
13 Sept. 1966	I	75	24	234	22	9.40	13	7	8.50
	II	75	15	290	15	5.16	10	3	4.43
	III	150	14	271	17	6.28	9	6	5.71
4 Oct. 1966	I	54	13	414	18	4.35	12	4	3.79
	II	54	11	453	7	1.54	4	1	1.00
	III	72	6	367	12	3.27	3	0	0.72
15 Nov. 1966	I	45	1	801	23	2.85	8	2	1.24
	II	45	0	932	19	2.04	2	0	0.25
	III	60	0	800	10	1.25	2	1	0.37

TABLE II. DAMAGE AND INSECT POPULATION DENSITY AT CONTROL PLOTS
(Preliminary experiment on cane fields [7])

Date	Plot No.	Total stem in sample furrows		Damage (%)	Total (In sample furrows)		Density
		Total number	Infested stem		Larvae	Pupae	
7 Sept. 1966	I	471	69	14.69	42	17	12.50
	II	346	24	6.74	15	5	5.76
	III	492	42	8.54	26	9	7.13
27 Sept. 1966	I	521	30	5.76	18	11	5.65
	II	420	20	4.79	12	7	4.50
	III	698	41	5.87	25	13	5.46
25 Oct. 1966	I	437	74	16.97	40	19	13.49
	II	574	59	10.22	27	18	7.83
	III	812	112	13.76	63	34	12.00
15 Nov. 1966	I	621	107	17.25	71	28	16.00
	II	519	65	12.50	33	31	12.27
	III	907	126	13.92	96	24	13.20

TABLE III. DAMAGE AND INSECT POPULATION DENSITY AT RELEASE PLOTS
(Last observation before harvesting)

Date	Plot No.	Rows			Total of stem		Total of		Total (Larvae + pupae)
		Letter	No.	Furrow	Total number	Infested stem	Larvae	Pupae	
14 March 1967	I	A	1	4	103	42	11	6	17
		B	3	14	84	19	7	2	9
		C	5	24	84	26	13	0	13
		D	7	34	82	29	12	4	16
		E	9	44	57	18	5	2	7
Total:					410	134			82
Damage (%)						32.4			
Population density									15.12
	II	F	11	2	90	39	14	4	18
		G	13	12	84	29	9	2	11
		H	15	22	66	28	11	1	12
		I	17	32	78	25	9	3	12
		J	19	42	83	22	6	0	6
Total:					401	143			59
Damage (%)						35.6			
Population density									14.71
	III	K	2	17	89	45	9	5	14
		L	4	27	87	24	6	3	9
		M	6	37	59	17	12	1	13
		N	8	47	71	26	3	3	6
		O	10	57	70	15	5	3	8
		P	12	67	78	21	6	1	7
		Q	14	77	87	41	15	2	17
		R	16	87	61	35	7	5	12
Total:					592	234			86
Damage (%)						37.8			
Population density									14.53

observation that took place on 25 October 1966 – they were higher by far than figures from the first and second tests: for damage 16.97%, 10.22% and 13.76%, respectively, and for population density 13.49, 7.83 and 12.00. The fourth observation took place on 15 November 1966; the amount of damage was 17.25%, 12.50% and 13.92%, while the population densities were 16.00, 12.27 and 13.20, respectively.

According to the figures obtained from the above mentioned observations, concerning both control plots and release plots, it is obvious that the second observation yielded decreased figures for damage and population density. Further observations yielded decreased figures for release plots and increased figures for control plots.

Before cane harvesting, observations were carried out once more (Table III). Observations at the release plots were performed on 14 March 1967 and gave the following damage figures: 32.4%, 35.6% and 37.8%, respectively, while the population density figures were 15.12, 14.71 and 14.53. Observation was carried out at the control plots on 24 March 1967 and yielded the following damage figures: 41.32%, 38.21% and 43.86%, respectively, while for population density the figures were 19.12, 16.07 and 19.27.

In this last observation before harvesting (Table IV), both method and object were the same as for the preceding ones. The amount of damage and the population density at the release plots were seen to be nearly the same as at the control plots. The only difference was in the appearance of the damage and it was obvious that the damage at the control plots was caused by a lingering, gradual infection, while the damage at the release plots was a new infection, which, for the greater part, was caused by a new infestation afflicting the cane tops in most cases.

4. DISCUSSION

Because of technical difficulties, this experiment was limited to studying the effect of sterile insect release upon attack intensity and population density. The data obtained were a means to compare the observation figures at both release and control plots.

The distance between the release plots and the control plots was about 5 km to avoid any effect caused by migration, since the maximum flight capacity of the white top borer moth is $\pm$ 1 km.

If the moths' flight capacity and the relatively small area of the release plots ($\pm$ 0.5 ha) are taken into consideration, the release of the sterile insects can be done in one only area of each plot. In our experiment the release was done in several areas to ensure that the entire plot would be covered.

Both male and female moths were released to provide a possibility for sterile female moths to mate with fertile males and thus reduce the probability of fertile males mating with fertile females.

Not all released pupae became moths. The highest death rate was found after the first release, partly because of the poor release technique and partly because of weather disturbances.

Because of the fact that there was no constant supply of pupae to be released, the number of sterile pupae did not always correspond with the estimated population, as is usually the case with such a release.

TABLE IV. DAMAGE AND INSECT POPULATION DENSITY AT CONTROL PLOTS
(Last observation before harvesting)

Date	Plot No.	Rows			Total of stem		Total of		Total (Larvae + pupae)
		Letter	No.	Furrow	Total number	Infested stem	Larvae	Pupae	
24 March 1967	I	A	2	12	92	12	4	4	8
		B	4	22	72	41	11	18	29
		C	6	32	103	41	14	12	26
		D	8	42	93	62	7	3	10
		E	10	52	95	32	6	8	14
Total:					455	188			87
Damage (%)						41.32			
Population density									19.12
	II	F	2	4	88	36	7	9	16
		G	4	9	86	28	4	5	9
		H	6	14	90	43	6	7	13
		I	8	19	85	32	3	9	12
		J	10	24	99	30	14	8	22
Total:					448	171			72
Damage (%)						38.21			
Population density									16.07
	III	K	2	8	103	34	12	9	21
		L	4	16	86	34	4	8	12
		M	6	24	98	33	6	11	17
		N	8	32	80	45	5	4	9
		O	10	40	90	56	6	7	13
		P	12	48	88	57	7	5	12
		Q	14	56	101	46	11	19	30
		R	16	64	117	34	9	16	25
Total:					773	339			149
Damage (%)						43.86			
Population density									19.27

Observations at release plots were only performed four times, but five times at the control plots; the fourth observation at the control plots was to ascertain whether there had been a repetition of the decrease in both population and attack intensity as had been noted after the second observation. The contrary was, in fact, observed; the fourth observation yielded increased figures, higher than those from any of the preceding tests on both fields, for both attack intensity and population density. The observations were carried out at different times because of the difference in the age of the sugar cane.

A complete mathematical calculation cannot be presented yet because of the lack of repeated experiments. For the time being it should suffice to say that we have comparatively increasing figures for control plots and decreasing figures for release plots where both damage intensity and population density are concerned.

5. CONCLUSIONS

The release of sterile insects caused a decrease in both attack intensity and insect population density.

The decrease of attack intensity at the release plots after the second observation was not so marked, but the decrease of the population density was obvious. This was partly because of the fact that the infested plants belonging to the first test were included in the second, while during the second observation there were not many new shoots.

The third observation at the release plots revealed a decrease in both damage and population density, while observation at the control plots showed an obvious and steady increase in these figures.

Since the experiment has only been carried out once within one growing period of the sugar cane, it will be necessary to repeat the experiment and to use improved release apparatus and more intense observation methods. A second test will soon be under way.

It has been proved that a sterile insect release can be simply and successfully carried out. If the second experiment yields positive results, the method can be put into practice soon on sugar cane plantations.

6. SUMMARY

Successful use of the sterile-male technique in controlling insect populations was reported in several countries. Certain characteristics of a candidate insect are important for the employment of this technique. Since 1965, experiments have been performed at the State Sugar Academy in Jogjakarta, Indonesia, to examine these characteristics in the white top borer (Scirpophaga nivella F.), a destructive sugar cane pest, and to determine the suitability of the sterile-male method. Several important characteristics were found suitable, namely, copulation ability did not decrease after radiation, and rearing on an artificial medium is possible for all life-cycle stages. Radiation at the pupal stage induced sterility in the adult.

Preliminary experiments in a sugar cane plantation were performed to determine the effect of released sterile insects upon attack intensity

and population density. The number of released sterile insects approximately equalled that of the natural population. Release was performed at a time when sugar cane was susceptible to attack in an area which had previously reported 10% annual losses due to the activity of this insect.

Released sterile insects caused a decrease in both attack intensity and population density. From the examinations carried out just before harvesting, after the release of the insects had been terminated, we found that the insect population density at the control plots as well as at the release plots was roughly equal. This fact led us to the conclusion that the release of the sterile insects will be effective in suppressing population increase if it is done constantly. There is another fact that supports this opinion, i.e. although the figures shown by the control plots and the release plots just before harvesting are almost equal, the damage in the release plots was to leaves only.

Thus, if we considered that damage caused by this pest to sugar cane 10 months old or older may be ignored, control could be accomplished by releasing sterile insects during the sensitive period of sugar cane growth. If, however, we want to destroy this pest completely, the release of the sterile insects must be done constantly during the growing period of the sugar cane.

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REFERENCES

[1] CHARPENTIER, L.J., MATHES, R., McCORMICK, W.J., SANFORD, J.W., Injury and Losses Caused by the Sugar Cane Borer in Louisiana, Entomology Research Division, Agricultural Research Service, USDA (1965).

[2] CLAUS, W.D., Radiation Biology and Medicine, Addison-Westly Publishing Company, Inc. (1958) 107-08, 688-700.

[3] DEVENTER, W. Van, De dierlijke vijanden van het suikerriet en hunne parasieten. Handboek ten dienste van de suikerrietcultuur en de suikerrietfabricage op Java 2, J.H. DE BUSSY Amsterdam (1912) 114-26.

[4] GLASSTONE, S., Sourcebook on Atomic Energy, D. Van Nostrand Co., Inc., New Jersey, Charles E. Tutle Company, Tokyo (1960).

[5] HATMOSOEWARNO, S., Rearing dan sexing Scirpophaga nivella F., Laporan tahap pertama "Team penggunaan radiasi atom untuk tanaman tebu" bidang hama dan penjakit. Kerdja sama antara P.G. Madukismo, Fakultas Pertanian U.G.M., Fakultas Biologi U.G.M., Fakultas Ilmu Pasti dan Alam U.G.M., Balai Penjelidikan Perusahaan Perusahaan Gula (B.P3.G.) Pasuruan dan Akademi Gula Negara Jogjakarta (1965).

[6] HATMOSOEWARNO, S., Kemungkinan penggunaan tenaga atom untuk pemberantasan hama penggerek putjuk putih (Scirpophaga nivella F.) pada tanaman tebu. Paper B-10, Simposium radioisotop I, Bandung, Indonesia (1966).
[7] HATMOSOEWARNO, S., Laporan tahap ke-empat "Team penggunaan radiasi atom untuk tanaman tebu" bidang hama dan penjakit. Kerdja sama antara P.G. Madukismo, Fakultas Pertanian U.G.M., Fakultas Biologi U.G.M., Fakultas Ilmu Pasti dan Alam U.G.M., Balai Penjelidikan Perusahaan Perusahaan Gula (B. P3. G.) Pasuruan dan Akademi Gula Negara Jogjakarta (1966).
[8] HATMOSOEWARNO, S., Atom dimasa damai dan kemungkinan penggunaan tenaga atom untuk pemberantasan hama penggerek putjuk putih (Scirpophaga nivella F.) pada tanaman tebu. Pidato Dies Natalis Akademi Gula Negara Jogjakarta, ke XVI (1966).
[9] KALSHOVEN, L.G.E., De plagen van de cultuur gewassen in Indonesia. N.V. Uitgeverij w. van hoeve's gravenhage, Bandung 1 (1960) 424-29.
[10] MARTORELL, L.F., MEDINA-GAUD, S., Status of Important Insect Pests of Sugar Cane in Puerto Rico and Their Control, Agricultural Experiment Station, University of Puerto Rico, Rio-Piedras, P.R. (1965)
[11] SANFORD, J.W., CHARPENTIER, L.J., MATHES, R., Chemical Control of the Sugar Cane Borer in Louisiana, Entomology Research Division, Agricultural Research Service, USDA (1965).
[12] Archief voor de suikerindustrie in Nederland Indie dan Mededelingen van het proefstation voor de Java suikerindustrie (1927 s/d 1934).
[13] USAEC, Radioisotopes in Science and Industry, A special report of the United States Atomic Energy Commission (1960) 12-14.

DISCUSSION

M. FRIED: Could you please indicate how large the treated plots were, how far distant they were from the untreated plots, and what was the ratio of sterile insects to native insects?

S. HATMOSOEWARNO: The treated plots were 0.5 ha each, and the distance between them and the untreated plots was about 2.5 km. The ratio of sterile insects to native insects was brought as close as possible to unity by calculation of the population density a week before release of the sterile insects.

H. ERDMAN: If you were to eradicate S. nivella would you expect greater damage from other insects? That is, on elimination of this component from the environment, will another species of insect pest become more destructive? I believe that the possibility of species replacement should be investigated.

S. HATMOSOEWARNO: I think that other insect pests causing damage to sugar cane plantations in Indonesia are not nearly so important as the white top borer, so that by controlling this pest we may reasonably hope for increased cane production. It should be borne in mind, however, that this first experiment of mine was simply a preliminary one covering only 1.5 ha, whereas by now I am working on a much larger area so that the possibility of another destructive pest assuming prominence can soon be thoroughly examined.

CONTROL OF ADULT PESTS BY THE IRRADIATION-OF-MALE METHOD*

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Abstract

CONTROL OF ADULT PESTS BY THE IRRADIATION-OF-MALE METHOD. The Grosch effect (i.e., of radiation interfering with utilization of nutrients in insects) became applicable to pest control when Galun and Warburg discovered that irradiated ticks fed once would seldom if ever feed again. This lifelong sensation of 'impletion' does not interfere with sexual competitiveness, but it does render harmless a pest that is a pest during the adult stage of its life cycle. It would seem that the general principle of treating adult pests to limit their feeding capacity could be extended to other pests. When insect pests are living in stored food products, it is a general rule that desirably low levels of radiation can be used to eradicate the pest if sterilizing doses rather than insecticidal doses are used. With the entire population of both males and females being irradiated, the sterilizing dose is often much lower than that which would be desirable when the insects are mass-reared for irradiation and release. Where mass-rearing techniques are not feasible, or where the pestiferousness of adults cannot be overcome by radiation or other treatments, field-irradiation facilities in baited traps can be devised. These have some advantages over similar traps containing chemosterilants. Wherever it can be used, the method of mass-rearing, irradiation, and release is still the most desirable way to control insect pests.

INTRODUCTION

The reliability of the irradiation-of-male method of insect control depends upon four effects of radiation [1], each of which must be studied for the insect under consideration, and each of which requires precise dosimetry. The first effect is that dominant lethal mutations are induced in the sperm by radiation. A dose of radiation must be used that induces dominant lethal mutations in more than half, and preferably upward of 99%, of the sperm. The second effect is that spermatogonial cells are killed by radiation. A dose of radiation must be used that is high enough to eliminate repopulation of the testes with normal spermatogonia by killing all of the spermatogonial stem cells. The third effect is that sperm are inactivated by radiation. A dose must be used that does not inactivate appreciable quantities of the sperm of polygamous species. For monogamous species, it does not matter whether the sperm are inactivated or whether dominant lethal mutations are induced. The fourth effect is that males are weakened by radiation. A dose of radiation must be used that does not debilitate the effectiveness of irradiated males when they are competing with normal males for the sexual attention of the females.

By a fortunate circumstance, for many insect pests the first two effects of radiation coincide at a dose of radiation below that at which the third and fourth effects become critical. Further, it is unnecessary to determine any of these or

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any other radiobiological parameters for females if the adult is not also a pest, unless there is discovered the unlikely insect where the oogonia are less sensitive than the spermatogonia and the females reproduce parthenogenetically.

IRRADIATION AND RELEASE OF ADULT PESTS

When only the female of a species is a pest, means for separation of the sexes can be invented, and the standard irradiation-of-male procedures can be used. The means of separation that have so ingeniously been devised comprise the physical (e.g., by utilizing weight, size, or osmotic differences), the genetic (e.g., marking the sex chromosome with a mutant gene for egg coloration), or the tropistic (e.g., using sex attractants). If both adults are pests, or the male alone is a pest, the problem takes on new dimensions.

Grosch [2] was the first investigator to demonstrate that the life-span was the same in starved and fed insects at doses above 100 kilorads. It could be inferred that in both cases the Habrobracon juglandis (Ashmead) females starved to death after being given large doses of radiation. The Grosch effect (i.e., of radiation interfering with utilization of nutrients) became applicable to insect control when Galun and Warburg [3] made the epochal discovery that irradiated ticks fed once would seldom if ever feed again, even at exposures of only 4000 R. This lifelong sensation of "impletion" rendered harmless a pest that is a pest when in the adult stage of its life cycle. Studies on other insects demonstrate that it is not simple to apply this principle universally. Little [4] has shown that the earwig, Chelisoches morio (F.), and the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), retained normal intestinal epithelium after they had been irradiated with doses of 10 kilorads. Destruction of intestinal cells became obvious at 100 kilorads. On the other hand, Riemann and Flint [5] showed that when the boll weevil, Anthonomus grandis Boheman, is exposed to 4000 R, the male-weakening from destruction of the intestinal tissues proceeds much too rapidly for the conventional irradiation-of-male procedures to be useful.

The exhaustive analyses by Riemann and Flint, the Grosch effect, and the phenomenon of Galun-Warburg impletion leave no doubt that killing the cells of the intestine destroys the capacity to utilize nutrients. Clark and Rubin [6] observed that Habrobracon serinopae (Ramkr.) females that were fed on a diet of honey and water died much later (92 days) than females given only paralyzed or unparalyzed larvae of their host Ephestia kühniella Zeller (40 days). When radiation was applied at increasing doses, the life-span decreased in both cases. The honey-fed wasps always lived longer than the Ephestia-fed wasps, but the percent decline at each dose was essentially identical. This indicates that nutrient utilization is declining in both cases to the same degree.

Work on mammals [7] has shown that rats become ravenous just before dying from radiation-induced intestinal injury because the blood sugar becomes depleted. The level of glucose in the blood triggers the sensation of hunger in mammals. That the phenomenon of Galun-Warburg impletion exists argues strongly for the necessity of physiological studies on insects to determine the mechanism of the hunger response. If irradiation of the intestine at doses suitable for insect control does not curb the feeding instincts of an insect as Little has shown, or if irradiation kills the insect at doses too low for insect control as Riemann and Flint have found, then some other treatment with or without radiation might bring on a permanent sensation of impletion; standard irradiation-of-male procedures

could then be applied. Understanding the origin and control of appetite and hunger in insects will open the way to using the irradiation-of-male method on any insect pest.[1]

IRRADIATION OF STORAGE PESTS

When the pests are living in stored food products, it would be preferable if relatively low doses of radiation were used. Radiation induces long-lived free radicals in grain, and the toxicity, carcinogenicity, and mutagenicity of these free radicals have not yet been adequately evaluated. Since lower doses give fewer free radicals, the lowest possible doses should be used to eliminate the storage pests. For this reason, sterilizing doses rather than insecticidal doses should be selected. Sterilizing doses generally are lower by far than insecticidal doses.

Nature is helpful too. The storage pest most suitable for the irradiation-of-male method would have the following characteristics: The eggs are more sensitive than the sperm. When these sensitive eggs are exhausted and the more resistant oocytes follow, then the spermatocytes that are much more sensitive than the sperm have matured to be sperm ready to fertilize the eggs coming from the resistant oocytes. A sterile period ensues for the male. By the time the spermatogonia have regenerated, the much more sensitive oogonia have rendered the females completely sterile. Since many insects conform to a pattern near to the ideal of alternating maxima of radiation sensitivities for the male and female, research with very low doses is worth trying for many insect pests. Further, the combined embryo survival from lacking dominant lethal mutations is a multiple of the sensitivities of the sexes at each stage of gametogenesis.

The principal storage pests that would give difficulty are the Lepidopteran species. As a rule, the Lepidopteran gametes are quite resistant to radiation [1], and the insecticidal doses range beyond 100 kilorads. Minimum sterilizing doses here well may be in the range of 20 kilorads or higher. At this level, one may indeed question the desirability of de-infesting foodstuffs with radiation. Another characteristic of Lepidoptera that in one respect makes it an organism of choice for the irradiation-of-male method is that adults of many species do not contain gonial cells, because gametogenesis is terminated during pupation.

The doses of radiation to infested grain can be made lower still if the grain were to be flooded with oxygen at the time of irradiation. This would enhance the radiation effect on the storage pests, probably without increasing the amount of free radicals in the grain.

IRRADIATION OF PESTS IN THE FIELD

Where mass-rearing techniques are not feasible, but where baiting can be easily achieved, then facilities for irradiation in the field can be developed. Such facilities to induce dominant lethal mutations have been developed for alkylating agents (chemosterilants). It would seem that radioisotopes could be as effective as chemosterilants in these traps. Even with strong β-emitting

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[1] The mechanisms underlying the phenomenon of hunger are beginning to be understood in some insects [cf. DETHIER, V. G., GELPERIN, A., J. exp. Biol. 47 (1967) 191-200].

isotopes such as strontium^{90} through its daughter yttrium^{90}, the traps would not need to have much more radiation shielding than a conventional chemosterilant trap. With γ-emitting isotopes, a considerable amount of radiation shielding would be required.

One advantage of using radioisotopes in bait traps is that they can be contained upon a surface in such a way that the insect would not carry any of the radioisotope away with him. Another advantage of using radioisotopes is that they are easy to find with ion chambers, whereas chemosterilants are not easily detectable. It is also likely that radiation-warning signs on the traps would be heeded because the public has become educated to the dangers of radiation over the last 20 years.

The disadvantages of such traps are the same as those for any trap or field-applied insecticide. Mutants of insects that are not attracted to the traps will be rapidly selected for in the population, and other possibly beneficial insects may go into the traps.[2] The best method is still to mass-rear, irradiate, and release.

OVERCOMING THE LIMITATIONS OF THE IRRADIATION-OF-MALE METHOD

When the irradiation-of-male method is ineffective, it is usually because the dose given is not high enough to prevent spermatogonial cell repopulation of the testes, or because weakening of males occurs at a dose below that required for inducing a high level of dominant lethality or gonial sterility.

The first case can be rectified simply by raising the dose of radiation. The second case presents difficulties which are by no means insoluble, but which might demand different solutions for different species.

If dominant lethality is too low when male-weakening becomes critical, then protracted (chronic) or fractionated doses of radiation can be given. In most insects, dominant lethal mutations in sperm are not repaired between fractionated doses of radiation or during protracted doses, whereas growing or dividing types of cells are repaired. In this way, high frequencies of dominant lethal mutations can be accumulated in the sperm and the animal will be stronger than when single acute doses are given.

If the dominant lethal test is made on the basis of hatchability, the criterion can be changed to adult survival since some of the dominant lethal mutations act during the larval and pupal stages. Spermatocytes seem always to be more sensitive than sperm; and by irradiating appropriate pupal stages, a greater sperm dominant lethality to male-weakening ratio might be achieved.

If the failure of the method lies with weakening of the males before gonial sterility is achieved, then protracted or fractionated radiation probably will not be helpful. This is because gonial cells are capable of repair, largely through the effect described by Kimball [8] where cells slowed in their rate of division by radiation have more time to repair their chromosomal damage before the mutations are rendered permanent by cell division.

Nonetheless, dominant lethality can be induced in spermatogonia [9] as well as oogonia [10] of certain insects. Thus, if the ratio of dominant lethal

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[2] These disadvantages are avoided by the method of capture, irradiation, and release developed by E. Horber (these Proceedings, SM-102/32).

mutations to total sperm from all matings over the lifetime of the male exceeds 0.50, the irradiation-of-male method will work despite spermatogonial regeneration. It is well known that radiation given at appropriate times during development will enhance the aging processes. It may be possible that irradiation with low doses at the right time of development could avoid male-weakening, would induce many dominant lethal mutations, and might enhance aging in a manner such that male-weakening could be established at approximately the same time that gonial cell repopulation of the testes takes place. Finding an appropriate "dominant lethal window" during development would require a lot of research effort, but it would be worth a lot of effort to develop the irradiation-of-male method to control, for example, the boll weevil.

Grell [11] has made an interesting suggestion based upon the observation of Edington [12] that the radioprotective substance AET does not protect Drosophila sperm from radiation-induced dominant and recessive lethality. Grell suggested that if the boll weevil could be induced to feed upon it, AET might protect intestinal tissue from radiation damage without affording protection to the spermatogonia or the cells in different stages of spermatogenesis.

Finally, as Knipling and others [13] have pointed out, if everything else fails, partial-body irradiation procedures could be developed and used to avoid the male-weakening problem that occurs with certain insects. Since partial-cell X-irradiation procedures have been developed for insect eggs by Ulrich and his colleagues [14,15], it should not be too large an engineering problem to automate the process for objects thousands of times larger.

Thus it would seem that with appropriate developments of myriads of techniques, any insect pest is suitable for selection to be driven virtually to extinction in infested areas by irradiating and releasing the pest. This assertion holds true for all but the thelytokous species where females produce female offspring parthenogenetically. Possibly even some of the thelytokous species may be controllable by release of irradiated females if the phenomenon of "flushing" described by Monro [16] operates effectively to drive out the pest simply by the pressure of adult populations demanding "Lebensraum."

CONCLUSION

The three keys to the widespread applicability of the irradiation-of-male method of pest control are that it can work [17,18,19], that it can work on any insect that reproduces sexually no matter how many times it mates [20,21], and that there is no limitation of the method to larval pests, for some pests that are pests as adults can be released after irradiation without their necessarily causing any damage [3]. This knowledge makes the irradiation-of-male method justifiable as a research that is all at once basic, applied, and current. It is an ideal type of project in radiation biology for investigators in countries where research funds are limited, because it is easy, inexpensive, and applicable to the often desperate needs of the country. Assistance from the United Nations is a necessity since an insect pays no attention to borders of countries and the warlike individuals on both sides of them. The individuals in bordering nations must often unite in their efforts to control the insect. As a peaceful use of atomic energy, research on the irradiation-of-male method most nearly fits the appellation of being truly international in scope.

REFERENCES

[1] von BORSTEL, R. C., Radiation and radioisotopes applied to insects of agricultural importance, IAEA, Vienna (1963) 367-385.
[2] GROSCH, D. S., J. Econ. Ent. 49 (1956) 629-631.
[3] GALUN, R., WARBURG, M., Use of isotopes and radiation in entomology, IAEA, Vienna (1968) in press.
[4] LITTLE, H. F., A. ent. Soc. Am. 60 (1967) 412-414.
[5] RIEMANN, J. G., FLINT, H. M., A. ent. Soc. Am. 60 (1967) 298-308.
[6] CLARK, A. M., RUBIN, M. A., Radiation Res. 15 (1961) 244-253.
[7] LINDOP, P. J., personal communication.
[8] KIMBALL, R. F., Repair from genetic radiation damage and differential radiosensitivity in germ cells (SOBELS, F. H., Ed.), Pergamon Press, London (1963) 167-178.
[9] ALEXANDER, M. L., Repair from genetic radiation damage and differential radiosensitivity in germ cells (SOBELS, F. H., Ed.), Pergamon Press, London (1963) 232.
[10] LaCHANCE, L. E., Radiat. Res. 11 (1959) 218-228.
[11] GRELL, E. H., personal communication.
[12] EDINGTON, C. W., Am. Nat. 92 (1958) 371-374.
[13] von BORSTEL, R. C., Technical Reports Series No. 44, Advances in insect population control by the sterile-male technique, IAEA, Vienna (1965) 71-73.
[14] ULRICH, H., Verhandl. Deutsch. Zool. Ges. in Hamburg 1956, 150-182.
[15] WÜRGLER, F. E., ULRICH, H., SCHNEIDER-MINDER, A., Repair from genetic radiation damage and differential radiosensitivity in germ cells (SOBELS, F. H., Ed.), Pergamon Press, London (1963) 101-106.
[16] MONRO, J., Technical Reports Series No. 44, Advances in insect population control by the sterile-male technique, IAEA, Vienna (1965) 22-24.
[17] BUSHLAND, R. C., HOPKINS, D. E., J. econ. Ent. 46 (1953) 648-656.
[18] BAUMHOVER, A. H., GRAHAM, A. J., BITTER, B. A., HOPKINS, D. E., NEW, W. D., DUDLEY, F. H., BUSHLAND, R. C., J. econ. Ent. 48 (1955) 462-466.
[19] KNIPLING, E. F., Science 130 (1959) 902-904.
[20] von BORSTEL, R. C., Science 131 (1960) 878, 880-882.
[21] von BORSTEL, R. C., BUZZATI-TRAVERSO, A. A., Radioisotopes and radiation in entomology, IAEA, Vienna (1962) 273-278.

DISCUSSION

D.S. GROSCH: I thank you for your recognition of my work. Perhaps I should explain why, in our work, little attention was given to the approach whereby radiation is used to interfere with the feeding of adult insects. At the time of publication of the report in the Journal of Economic Entomology much attention was being focussed on control of stored grain pests. However, pure food and drug laws in the United States condemn any grain or flour in which parts of insects, such as legs, wings, tergites, sternites and so on, are found, and therefore this approach was considered

impractical, but now that it is proposed to apply this technique to ticks, its future use seems promising.

R. C. VON BORSTEL: I'm pleased that you too are optimistic on this score.

V. LABEYRIE: The females of Oryzaephilus surinamensis (Coleoptera: Cucujidae) oviposit over a period of several months; virgin females do not oviposit; the males induce ovipositing by mating. Females mating with non-virile males lay eggs which are sterile, but if, after 30 days of non-productive ovipositing, these females are put together with virile males they are found to lay fertile eggs. This shows that a second mating can cause ovipositing of fertile eggs when the first dose of spermatophore is incapable of fertilization. It is thus quite feasible that females first mated with males whose sperm has been destroyed by irradiation may yet be fertilized by normal males.

R. C. VON BORSTEL: Thank you for this information. Dr. Gomez-Nunez told me of his evidence with Rhodnius indicating that inactivated sperm cannot compete with normal sperm, but it is certainly known from work on several other species that 'lethal' sperm do compete.

D. T. NORTH: I would like to comment on several of the points you made. First, when considering the possible use of aspermic males to reduce the reproductive potential of a population of a polygamous species, it must be remembered that these can only reasonably be effective where the density of population is such that the average number of matings per female is low, i. e. 1.0 or below. In other words, the effectiveness of a released sterile male is dependent on the probability of his being able to reduce the reproductive potential and this varies with each type of population.

Secondly, I should like to mention that data do exist on the problem of radiation-induced destruction of the gut in the boll-weevil. When dealing with an insect species in which the adult causes destruction, a short-lived and effective sterile male may well be an advantageous proposition. I should also like to point out that your suggestion on the use of radioprotective agents like AET has been put to the test in our laboratory by Dr. H. M. Flint, with no success. He found, furthermore, that by virtue of its mode of action a protective agent may well protect the sperm from induction of dominant lethals, thereby leaving the problem unsolved. With regard to your proposal to shield the gut whilst irradiating the testes I refer you to a paper by Riemann and Flint (Riemann, J. G. and Flint, H. M., Irradiation effects on midguts and testes of the adult boll weevil, Anthonomus grandis, determined by histological and shielding studies, Ann. ent. Soc. Am. 60 (1967) 298-308) published in 1967, in which they demonstrated this effect and showed that the gut was in too close a proximity to the testes for the method to be practicable.

F

RADIATION EFFECT STUDIES: GENETIC

(Session VIII, Part 1)

Chairman: Ø. STRØMNAES
NORWAY

RADIATION SENSITIVITY AND REPAIR OF CHROMOSOMAL DAMAGE

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Abstract

RADIATION SENSITIVITY AND REPAIR OF CHROMOSOMAL DAMAGE. If the sterile-male technique in pest control is used, it is important for irradiated sperm to have a dominant lethal effect. In Drosophila, sperm and spermatogonia are radiation resistant cells compared with spermatids and spermatocytes. After a moderate radiation dose, the male might be fertile for several days and then go through a sterile period. Fertility is regained when cells that were spermatogonia during the time of irradiation are available for fertilization. It is therefore important for the irradiation dose to be lethal to all spermatogonia so that no repopulation of spermatogonial cells can occur. It should be possible to find a radiation dose which renders insects sterile without impairing their mating behaviour and vitality. The radiation dose can also be adjusted by changing internal or external environments during irradiation.

1. INTRODUCTION

Our knowledge of mutagenicity has greatly increased in the 40 years that have elapsed since Muller [1] discovered that ionizing radiation induces mutation. A little is known about how chemical mutagens act at the molecular level, and we have some idea how ultra-violet radiation produces mutations. With regard to ionizing radiation, our knowledge of the molecular changes that lead to inter- and intragenic changes is still very limited. Among the many unsolved problems is, for example, the molecular structure of chromosomes in eucaryotes, whether they consist of single or multi-stranded DNA, and how the nucleoproteins are arranged.

2. STRAIN DIFFERENCES IN RADIATION SENSITIVITY

Radiation sensitivity varies greatly between organisms, strains, cell types, and cells in different stages of development. Ogaki and Tanaka [2], who studied the survival rate of various Drosophila strains, found the LD_{50} for females in two wild type strains to be about 150 000 R, while for the females in two mutant strains it was 120 000 R. The LD_{50} for males was somewhat lower, 130 000 and 100 000 R, respectively. Radiation resistance was shown to be dominant, and mostly controlled by the third chromosome.

The mutability of 13 related strains and 17 unrelated strains of Drosophila melanogaster exposed to X-ray treatment was investigated by Strømnæs [3]. He found significant differences in mutability between strains, and it was suggested that some strains have genotypes extra-sensitive to irradiation, and that there are both fairly homogeneous and heterogeneous strains in this respect. These data are strongly supported by data published by other investigators [4-6]. Further tests were performed by Strømnæs [7], who made 11 repeats over a period of 4 years at two different institutes on two different strains, Iso-Amherst and Oslo.

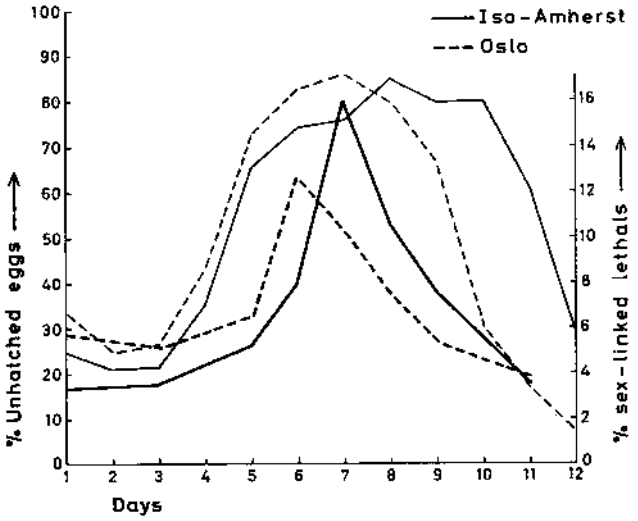

FIG. 1. Sensitivity patterns for two wild type strains of Drosophila melanogaster. The upper two curves represent dominant lethals (Strømnæs [8]), and the lower two curves represent recessive sex-linked lethals (Ward and Bird [13]).

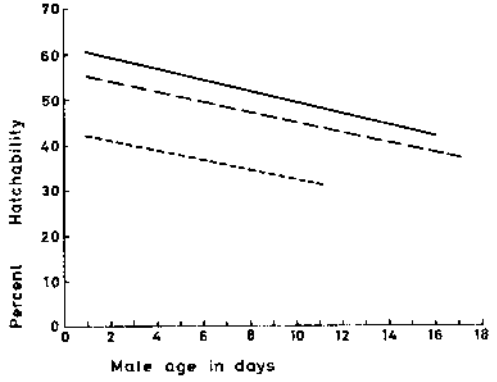

FIG. 2. Hatchability of eggs fertilized by sperm from males irradiated at different ages. The lines indicate the general trend given in the data by ———— Dempster [14], ------ Strømnæs [15], and ----- Bonnier and Lüning [16].

The higher mutation frequency was observed in the Oslo strain, and it was later shown [8] that the two stocks also have different brood patterns.

That a difference in radiation sensitivity also exists between strains of Escherichia coli has been demonstrated by Hill [9]. She showed that Strain B_S is much more sensitive to X-rays than Strain B. Strain B_S was derived from Strain B by u.v. treatment, and it is therefore most likely that the difference in sensitivity to X-rays is caused by a u.v.-induced mutation. In yeast, homozygosity for the mating-type gene leads to higher sensitivity to ionizing radiation than heterozygosity [10], and there are isolated radiation-resistant [11] and radiation-sensitive mutants [12].

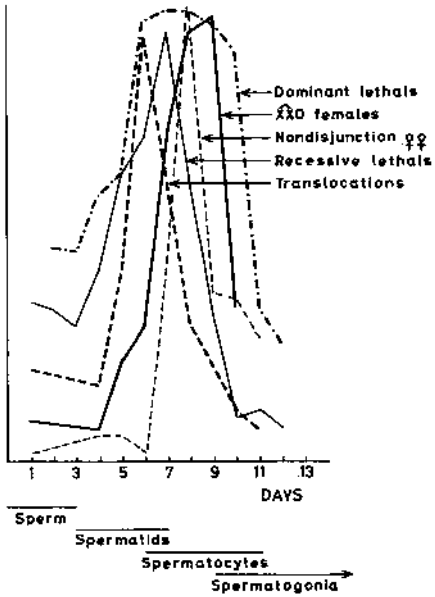

FIG. 3. Sensitivity patterns for different types of radiation effects in sperm available for fertilization on various days after irradiation of Drosophila melanogaster males. Dominant lethals (Lüning [21]), ♂XO females (Sävhagen [35]), non-disjunction females (Sävhagen [29]), recessive lethals (Ives [28]), translocations (Sävhagen [35]). The ordinate is the relative frequency of each radiation effect normalized to approximately the same value at all peaks (from Sävhagen [104]).

Ward and Bird [13] irradiated males of the same two stocks as Strømnæs [8] had used, and scored the rate of sex-linked recessive lethals on days 1, 3, 5, 6, 7, 8, 9 and 11 after irradiation. Their data strongly supported those found by Strømnæs (Fig. 1). Ward and Bird also investigated cytochrome C oxidase activity and found it to give two different patterns in the two strains. Their conclusion is that the difference in radiation sensitivity between the two strains cannot be explained on the basis of a protective effect of the cytochrome system alone. To explain the entire sensitivity pattern of the two strains, the operation of two other factors besides the enzyme mentioned is necessary.

Thus, different strains can have different sensitivity to irradiation, which may also be reflected in their brood pattern. Investigations by Dempster [14], Strømnæs [15], and Bonnier and Lüning [16] demonstrate that the mutation frequency is dependent on the age of the male at the time of irradiation (Fig. 2). The sensitivity increases with increasing age of the male. A similar effect was found when eggs were aged [17].

Bonnier and Lüning [16] also observed a decrease in hatching rate with increase in time from irradiation to fertilization.

3. RADIATION SENSITIVITY DURING SPERMATOGENESIS

Harris [18] employed mating periods of 4 days and found a drop in sex-linked lethals in the brood coming 12 to 16 days after irradiation.

Auerbach [19] started using constant mating periods of 3 days and found the highest rate of sex-linked recessive lethals in brood C, which is in good agreement with Lüning's [20] observation.

3.1. Dominant lethal mutations

With the introduction of 24-hour mating periods a much more detailed picture was obtained of the radiosensitivity of the different cell stages of spermatogenesis. The lack of hatchability of eggs fertilized by irradiated sperm has usually been referred to as dominant lethals. From such studies it was evident that from the 5th day after irradiation there is a marked increase in the frequency of dominant lethals, with a maximum between the 6th and 10th day followed by a decrease on the 11th day [18, 21, 22, 23, 24, 25] (Fig. 3).

3.2. Recessive lethal mutations

For other types of induced genetic damage such as sex-linked lethals [26-28], an increase in frequency is observed from the 4th day with the peak reached 6 to 7 days after irradiation, followed by a sharp decrease on the 8th day. A similar sensitivity pattern is reported for autosomal recessive lethals [26].

3.3. Non-disjunction

Still another method of studying induced genetic damage is to study losses of paternal sex chromosomes and non-disjunction as a simultaneous loss of paternal X and Y chromosomes. Sävhagen [29] performed such a study and found non-disjunction to be highest in sperm available for fertilization 7 to 10 days after irradiation. She concluded that these cells had been in early meiosis, prior to anaphase I, at the time of irradiation.

3.4. Recombination

Another way of determining meiosis is by studying the products of crossing over in successive broods. If crossing over occurs in first meiotic division the recombinants should occur singly, but occur in bundles if they are the result of mitotic crossing over. Sävhagen [30] found only single recombinants in sperm ready for fertilization on the 7th and 8th day after irradiation, mostly singly, but occasionally also bundles of identical recombinants on the 9th to the 11th day, and on later days clusters of crossovers were frequently observed. This means that sperm available for fertilization from the 12th day on must have been irradiated as spermatogonia. The conclusion is in general agreement with observations made by other investigators [26, 28, 31, 32, 33, 34].

3.5. Translocations

For autosomal translocations, very much the same sensitivity pattern has been found as for sex-linked recessive lethals. Thus, the peak is reached on the 6th day followed by a sharp drop on the 8th day after irradiation [27, 35, 36].

3.6. Conclusion

From sensitivity patterns of the types mentioned, the conclusion can be drawn that sperm available for fertilization the first three days after irradiation of mature males must have been mature sperm at the time of irradiation. Sperm available in the next two days must have been irradiated as spermatids, and the sperm available from the 6th to the 10th day must have been spermatocytes; sperm coming on later dates must have been irradiated as spermatogonia.

The brood pattern may vary somewhat depending on the strain used, the age of the males, the mating procedure, and perhaps the irradiation dose [37]. Besides, there are variations between individual males; and the possibility exists of mixing of sperm from different stages of development during irradiation. Thus, sperm sampled 8-10 days after irradiation may have been irradiated either as spermatocytes or spermatogonia.

4. SENSITIVITY OF SPERMATOCYTES

A special study of induction of mutations in spermatocytes was performed by Chandley [38]. She found a high frequency of recessive sex-linked lethals on days 5, 6 and 7 after irradiation and then a low frequency on day 8. For translocations the high rate was observed 5 and 6 days after irradiation with a decrease on day 7 and a low level on day 8. The highest frequencies of dominant lethals were observed on days 6, 7 and 8 after irradiation. The induced frequency of crossing over and hyperploid females were low on day 5 and increased on later dates, reaching peak value on day 8. Her data and interpretations are in general agreement with those published by other authors, and also her conclusion that: "one should never talk loosely of a 'sensitive' or 'insensitive' stage without indicating what type of mutation is under consideration."

5. SPERMATOGONIAL SENSITIVITY

A low frequency of mutation is observed in sperm irradiated as spermatogonia. Mutations involving gross chromosomal aberrations will most likely be eliminated during meiosis. Oakberg [39, 40] found the chief radiation effect to be killing of most of type A spermatogonia and all intermediates and type B spermatogonia. Oftedal [41] suggested that for Drosophila two spermatogonial components exist that have different mutability and viability sensitivity in regard to X-rays. The salivary glands of successive broods of irradiated males were studied by Slizynska [42]. She looked for changes such as translocations, inversions, deficiencies, repeats, duplication, and loss of the 4th chromosome. All males seem to exhibit the same sensitivity when the sperm and spermatid stages are irradiated. However, the spermatogonial stage discloses a heterogeneous population of males; some have spermatogonia that are extra-sensitive to X-rays, while others have spermatogonia that are insensitive. Slizynska explains this variation in sensitivity in spermatogonia by the presence or absence of spermatogonial mitosis.

McCarthy and Nafei [43] found no males with extra-sensitive spermatogonia. That spermatogonia in mice constitute a heterogeneous popula-

tion has been verified by Nebel, Coulon and McWhinnie [44]. They found that spermatogonia of type B are more sensitive than type A, but within the latter the stages G_1 and S are extra-sensitive. Thus, it may be more correct to talk about a population of cells with sensitive stages in a normal cell cycle, than to talk about a heterogeneous population of cells.

6. SPERM SENSITIVITY

6.1. Within males

Sperm available for fertilization on the first two days after irradiation has been studied intensively. It has been known for some time [45] that when males 3-4 days old are irradiated, the second-day sperm has a lower mutation frequency than the first day sperm. This led to a discussion of whether differential sensitivity existed, or if recovery from damage of the genetic material occurred from one day to the next [46-50]. The conclusion reached by Nordback and Auerbach [51] was that recovery occurs from intergenic as well as intragenic damage to the genetic material. Traut [52] comes to another conclusion, namely that differential sensitivity exists rather than recovery of genetic damage from one day's sperm to the next. This view is also held by others [53, 54], who believe that mixing of sperm of different sensitivity can occur in testes after irradiation. Müller [55] supports this view and says that the mixing groups of sperm are characterized by different degrees of oxygenation, an interpretation that is credited to Oster. Muller also reviews pertinent papers by others.

In discussions on induced mutation frequency in first-versus second-day sperm, the age of the male at the time of irradiation is of the utmost importance. Khishin [56, 57] demonstrated that the first motile sperm in males of the Oregon-K stock was observed 7 ± 1 hours after eclosion. Strømnæs and Kvelland [58] studied in more detail the sexual activity of Drosophila males, and found that older males are capable of mating more times than younger males in a twelve-hour period. The older males are able to produce more offspring than the younger ones as well as a larger total per fertile mating. Furthermore, a 12-hour celibacy period is sufficient to bring the amount of sperm available for fertilization up to the amount available in males of the same age but previously not mated. (For more details see also Refs [59-63]). That second-day sperm may have a different sensitivity to irradiation than first-day sperm seems to be brought out by the data published. However, when it comes to males withheld from mating for one day, mixing of first- and second-day sperm should be more pronounced in young than in old males.

If single ejaculates are studied, much more detailed information about mutation sensitivity in mature and nearly mature sperm should be obtained. Mossige [64] performed an experiment where mating was observed and the frequency of mutation in successive ejaculates studied. She found that the frequency of sex-linked lethals decreased in consecutive matings for a period up to about 24 hours when 0-4-hour-old males were irradiated. For 2-day-old males there was a decrease in frequency with time up to 9 hours. In 7-day-old males there was a decrease from first to second ejaculate and no further decrease. Mossige irradiated the males in air,

oxygen and nitrogen, and from these data she concluded that the decrease observed is due to a difference in oxygen sensitivity and to recovery with time for mature sperm from males up to 2 days old. Strømnæs and Kvelland [65, 66] studied the frequency of induced Minutes, sex-linked lethals, and translocations in successive ejaculates during two twelve-hour periods separated by a twelve-hour male celibacy period. The males at the time of irradiation were 0-2, 12, 24, and 72 hours old. For sex-linked lethals and translocations there were significant differences in mutation frequency between male age groups in the first 12-hour mating period after irradiation, while the variation between age groups in the second mating period was insignificant. Furthermore, by testing successive sperm ejaculates, it was possible to determine the time when sperm of less sensitivity became available. This was found to be available earlier in very young males than in older males. Similar results were obtained by Lüning [67] who found that a change in mutation frequency occurred after 4 hours for males irradiated when 0-24 hours old, while no change in mutation frequency was found the first 12 hours after irradiation of 3-4-day-old males (see also Ref. [64]).

Differential mutability and recovery from irradiation damage in the different male germ cell stages and individual ejaculates have also been discussed by Lefevre and Jonsson [68] and Lefevre [69].

Whether differential sensitivity and/or recovery with time operate in mature or almost mature sperm is difficult to determine, since ejaculate sequence is not independent of time. However, we have now a substantial amount of data which are being tested by partial correlation analysis, and we hope the result will shed more light on this question.

If recovery is going on in male germ cells and is controlled by a protein, lowering the metabolism should affect the induced mutation rate. This problem was studied by Wedvik and Strømnæs [70] and Sollunn and Strømnæs [71]. They found certain changes in the brood pattern for 3-day-old males. Thus, the frequency of dominant lethals is higher in the seven first broods for the males irradiated at 7° C than for those irradiated at 22° C, and no statistically significant differences were observed for later broods.

When irradiation is performed at 0° C and 22° C, brood comparisons for the three first broods always reveal a higher frequency of dominant lethals and recessive sex-linked lethals at 0° C than at 22° C. Variation between first and second brood is significant for sex-linked lethals at both temperatures and only at 22° C for dominant lethals. These results are compatible with a repair enzyme system that is operative in sperm and spermatids at room temperature but is inactivated at 0° C. Apparently, the enzyme acts on the breakage restitution process (dominant lethals), and does not affect noticeably recessive sex-linked lethals.

6.2. Within females

Bonnier and Lüning [72] were the first to observe that sperm irradiated in females is more sensitive to irradiation than sperm treated in the male. Data supporting this finding were published by Abrahamson and Telfer [73]. Traut [74] found that irradiation of inseminated females gave no higher mutation frequency in the sperm than was found after irradiating sperm in the male, and Lefevre [75] came to the conclusion that sperm irradiated

in the male exhibits a detectably higher mutation frequency than that irradiated in the female. It was believed [76] that when sperm is irradiated in the male, pre-existing detrimentals may act synergistically with induced ones to give completely lethal chromosomes, while this effect is absent with sperm irradiated in the female. However, more recent experiments by Lefevre [77] show that there is a more highly significant reduction in mutation frequency in the female genome when both paternal and maternal genomes are irradiated than if only the female genome is irradiated. This is valid for mutations discovered in eggs laid the first four days after irradiation, but not for eggs laid later, which indicates that in relatively mature germ cells there is preferential elimination of chromosomal aberrations by an interaction of the two irradiated genomes.

Lefevre [78] reinvestigated the effect of irradiation of fully mature sperm in the male and in the female. He found a reduced frequency of hyperploid males and sex-linked recessive lethals when inseminated females rather than males were irradiated. Lefevre favours the idea that the difference in observed mutation frequency is caused by elimination of some zygotes when both genomes are irradiated, and sees no reason why the sperm should have a different sensitivity in the two environments or should be preferentially repaired when irradiated in the female.

7. ALTERATION OF THE ENVIRONMENT

Radiosensitivity can be influenced by alterations in external and internal environment before, during or after irradiation. Alterations of this type also influence the mechanism of repair from genetic damage. Several review papers and books on the subject have been published lately [79-82]. Repair and radiosensitivity phenomena in Drosophila were studied in considerable detail by Sobels and coworkers (see reviews by Sobels [83-85]). One of the interesting findings is that when Drosophila males are exposed to a high dose-rate of X-rays under anoxia, a reduction in the frequency of recessive lethals and translocations is found in spermatozoa when the post-treatment is N_2, but when the post-treatment is O_2 the reduced frequency is observed in early spermatids. The same result is obtained when spermatozoa are irradiated in females or spermatids in 24-hour-old pupae. It is suggested that the post-radiation effects in both types of cells arise from enzymatic repair of potential lesions. Sobels thinks that in sperm both glycolytic enzymes and RNA or protein synthesis are involved in the repair process. For repair to occur in early spermatids, oxygen is needed, and a reduction in mutation frequency is observed when inhibition of RNA and/or protein synthesis occurs.

Dickerman [86] studied the induction of dominant lethals by irradiating Drosophila virilis oöcytes in different gases. The gases used were air, argon, helium, methane, carbon monoxide, and oxygen. He found that the frequency of dominant lethals induced in Stage 7 and Stage 14 oöcytes increased with increased oxygen tension during irradiation. The data indicated a protective effect of argon, methane, and helium. Since methane is a lipid soluble gas, it is suggested that the oxygen site is a lipid fraction of the cell. Dose effect curves showed that Stage 7 oöcytes are much more resistant than Stage 14 oöcytes. The explanation given for this is that Stage 7 oöcytes are metabolically active and able to repair chromosomal

damage in a short time, while Stage 14 oöcytes are less active, but when fertilized, go rapidly through meiosis whereby chromosome movements make restitution less likely.

Induced dominant lethality in oöcytes has also been studied by Rinehart [87]. His conclusion is that in Stage 7 oöcytes there is an oxygen-dependent repair system. The repair system is degraded in the absence of oxygen, but regenerates again when oxygen is administered. Rinehart thinks this repair system is inoperative in Stage 14 oöcytes until after fertilization. This, together with chromosome movements, is part of the explanation why sensitivity is greatly increased in Stage 14 oöcytes as compared with Stage 7 oöcytes.

8. THE BASIC LESION AND ITS REPAIR

Information about radiation sensitivity and repair can also be obtained by employing dose fractionation. One might expect that the mutation frequency would be the same for the same total dose no matter how the dose is delivered. However, Sax [88, 89] showed that the yield of chromosome aberrations for low LET given in two equal fractions depends on the time interval between the fractions. The usual explanation is that radiation-induced breaks have a certain lifetime within which they can interact and produce exchanges. If there is not a second break within rejoining distance the break may restitute. Thus, as the time between radiation fractions is extended fewer breaks will be available for the formation of exchanges.

Savage [90] distinguishes between 3 types of sites which can have 2, 1 or no exchange lesions. Exchange can take place at sites with 2 lesions, while sites with one lesion may undergo restitution. This will lead to diminished interaction which can also be achieved by inactivation of sites. Savage found that the yield of chromosome exchanges is less than simple additivity of the yield of the two doses given independently, when the second dose is given at a time when exchanges with lesions produced by the first dose are minimal. This is consistent with the idea of repair or restitution of potential exchange lesions, but the data cannot exclude the possibility of dose-dependent inactivation of sites.

Evans [91] presents the hypothesis that radiation does not produce a direct breakage of chromosomes, but induces lesions that are initially unstable. These lesions become stabilized, and, some hours after irradiation, undergo repair. The repair may be analogous to that found in certain micro-organisms exposed to ultra-violet light or alkylating agents and may involve the excision of the damaged zone and the resynthesis and polymerization of new chromosome materials. Chromosome aberrations are therefore suggested to be a consequence of mis-repair (cross polymerization) involving two lesions. Savage suggested that regions that have not undergone repair in interphase appear at mitosis as non-staining gaps in the chromatids. Evidence is presented that single or paired gaps showing linear and dose-squared kinetics are repairable lesions, and that the majority of gaps are repaired as a consequence of DNA synthesis.

The best evidence published so far that irradiation breaks chromosomes by breaking the DNA has been published by von Borstel, Miller and Carrier [92, 93]. They irradiated amphibian lampbrush chromosomes in

intact oöcytes, in isolated nuclei, and irradiated the isolated chromosome sets. In this way, they were able to demonstrate that chromosome breaks are induced by direct action of X-radiation rather than via some cell structure.

Kirby-Smith and Randolph [94] suggested that X-ray-induced damage is in the protein part of chromosomes, while u.v. damage is in the DNA. Furthermore, data presented by Chu [95] also indicate that protein synthesis is necessary in repair of chromosome damage. Revell [96] advanced the idea that the chromosomes get activated spots, which, when located close enough to other such spots, break and rejoin to form exchanges. Wolff [97] disagrees with Revell on the point of activated spots, and believes an actual break is produced at the time of irradiation. With regard to restitution, Wolff [98] finds that fractionation of dose with a protein synthesis inhibitor present will inhibit the restitution of chromosome breaks in the interval between radiation doses. FUdR, which inhibits DNA synthesis, has no effect on the restitution process. Wolff [99] says that repair occurs in those parts of the cell cycle where no DNA synthesis takes place, and that the bonds formed in chromosome rejoining are proteinaceous. Thus, there seems to be substantial evidence that restitution of breaks is dependent on protein synthesis. It has not been demonstrated that DNA synthesis is going on at the same time, but this cannot be completely excluded.

Since restitution is dependent on protein synthesis, it is likely that defects in proteins may lead to chromosome breaks. It is known that the metabolic inhibitor parafluorophenylalanine (pFPA) can be built into proteins. A recent study by Strømnæs [100] of the mutagenic effect of pFPA in Saccharomyces shows that pFPA induces crossing over, translocation, inversion and monosomics. The effect of pFPA is believed to be through incorporation in proteins necessary for chromosome integrity and distribution or DNA duplication.

Some interesting preliminary data on the effect of different doses of irradiation on the survival of yeast cells have been obtained by Strømnæs and Mortimer (unpublished). These data show that for low dosages there is a drop in survival frequency, while higher dosages give a higher survival frequency until such dosages where the survival frequencies again decrease. The explanation may be that a certain irradiation dose is necessary to activate a radiation-protective enzyme system. Thus, linearity for low doses would not be expected. Calkins [101] operates with two types of recovery systems of which one, the T system, does not operate after low doses, but does operate at higher doses, and may thus be similar to what we believe to have found. Calkins says that activation of the T system requires radiation damage or some equivalent stimulus. If a radiation dose of a certain size is necessary to stimulate a repair enzyme system, this may be the explanation why Shiomi [102] finds no significant difference between first- and second-day sperm treated with 1000 R, but observes a significant difference after treatment with 2000 R, 3000 R, and 4000 R. He studied sex-linked lethals and translocations. Sankaranarayanan [103] found no significant difference between nitrogen and oxygen post-treatment in the frequency of dominant lethals induced by 4000 R of X-rays under anoxia. Sankaranarayanan thinks only a small fraction of the dominant lethal damage is capable of being repaired, while the major portion is irreparable.

REFERENCES

[1] MULLER, H.J., Science 66 (1927) 84.

[2] OGAKI, M., TANAKA, E., Genetics 48 (1963) 904.

[3] STRØMNÆS, Ø., Hereditas 37 (1951) 533.

[4] DUBOVSKY, N.V., C.R. (Dokl.) Acad. Sci. U.R.S.S. 4 (1935) 95.

[5] DEMEREC, M., Genetics 22 (1937) 469.

[6] DEMEREC, M., KAUFMANN, B.P., HOOVER, M.E., The gene. Ann. Rept. Dept. Genetics, Carnegie Institution of Wash. 1937/1938 (1938) 40.

[7] STRØMNÆS, Ø., Progress in Radiobiology, The Kynoch Press, Birmingham, (1955) 196.

[8] STRØMNÆS, Ø., Hereditas 45 (1959) 221.

[9] HILL, R.F., Biochim. Bioph. Acta 30 (1958) 636.

[10] LASKOWSKY, W., Z. Naturforsch. 15b (1960) 495.

[11] MOUSTACCHI, E., Mutation Res. 2 (1965) 403.

[12] NAKAI, S., MATSUMOTO, S., Mutation Res. 4 (1967) 129.

[13] WARD, C.L., BIRD, M.A., Genetics 47 (1962) 99.

[14] DEMPSTER, E.R., Proc. nat. Acad. Sci. 27 (1941) 366.

[15] STRØMNÆS, Ø., Genetics 34 (1949) 462.

[16] BONNIER, G., LÜNING, K.G., Hereditas 36 (1950) 445.

[17] PATTERSON, J.T., BREWSTER, W., WINCHESTER, A.M., J. Hered. 23 (1932) 325.

[18] HARRIS, B.S., J. Hered. 20 (1929) 299.

[19] AUERBACH, C., Cold Spring Harbor Symp. Quant. Biol. 16 (1951) 199.

[20] LÜNING, K.G., Acta zool. 33 (1952) 193.

[21] LÜNING, K.G., Hereditas 38 (1952) 91.

[22] STEGER, R., Oncologia 9 (1956) 12.

[23] STONE, W.S., Mutation, Brookhaven Symp. in Biol. 8 (1956) 171.

[24] BATEMAN, A.J., J. Genetics 54 (1956) 400.

[25] FRITZ-NIGGLI, H., Bull. schweiz. Akad. med. Wiss. 14 (1958) 550.

[26] AUERBACH, C., Zeitschr. f. Ind. Abst. u. Vererb. lehre 86 (1954) 113.

[27] SOBELS, F.H., Adv. radiobiol. (DE HEVESY, G.C., FORSSBERG, A.G., ABBATT, J.D., Eds.), Oliver and Boyd, London (1957) 449.

[28] IVES, P.T., Int. J. Rad. Biol. 2 (1960) 54.

[29] SÄVHAGEN, R., Hereditas 47 (1961) 43.

[30] SÄVHAGEN, R., Hereditas 47 (1961) 23.

[31] SOBELS, F.H., Zeitschr. f. Ind. Abst. u. Vererb.lehre 87 (1956) 743.

[32] KVELLAND, I., Hereditas 48 (1962) 221.

[33] HANNAH-ALAVA, A., Mutation Res. 1 (1964) 414.

[34] PURO, J., Annales Zool. Fennici 3 (1966) 99.

[35] SÄVHAGEN, R., Hereditas 46 (1960) 651.

[36] CLARK, A.M., Amer. Natur. 89 (1955) 179.

[37] FAHMY, O.G., FAHMY, M.J., Mutation Res. 1 (1964) 247.

[38] CHANDLEY, A.C., Int. J. Rad. Biol. 5 (1962) 305.

[39] OAKBERG, E.F., J. Exptl. Zool. 134 (1957) 343.

[40] OAKBERG, E.F., Rad. Research 11 (1959) 700.

[41] OFTEDAL, P., Genetics 49 (1964) 181.

[42] SLIZYNSKA, H., Genet. Res. 5 (1963) 248.

[43] McCARTHY, J.C., NAFEI, H., Genet. Res. 4 (1963) 485.

[44] NEBEL, B.R., COULON, E.M., McWHINNIE, D.J. Cytol. 28 (1963) 81.

[45] BAKER, W.K., VON HALLE, E.S., Proc. Natl. Acad. Sci. 39 (1953) 152.

[46] LÜNING, K.G., Hereditas 44 (1958) 161.

[47] LÜNING, K.G., HANNERZ, B., Hereditas 43 (1957) 550.

[48] LÜNING, K.G., HENZE, A., Hereditas 43 (1957) 572.

[49] LÜNING, K.G., SÖDERSTRÖM, J., Hereditas 43 (1957) 564.

[50] LÜNING, K.G., SCHUWERT, B., JONSSON, S., Hereditas 44 (1958) 170.

[51] NORDBACK, K., AUERBACH, C., Advances in Radiobiology (DE HEVESY, G.C., Ed.) Oliver and Boyd, Edinburgh (1957) 481.

[52] TRAUT, H., Genetics 50 (1964) 167.

[53] TROSKO, J.E., Genetics 49 (1964) 401.

[54] TROUT, W.E., Genetics 50 (1964) 173.

[55] MULLER, H.J., Genetics Today (GEERTS, S.J., Ed.) Proc. XI Int. Congress Genetics, Hague 1963. Pergamon Press, London (1965) 265.

[56] KHISHIN, A.F.E., Zeitschr. f. ind. Abst. - u. Vererb.-l. 87 (1955) 97.

[57] KHISHIN, A.F.E., Cytologia 22 (1957) 28.

[58] STRØMNÆS, Ø., KVELLAND, I., Hereditas 48 (1962) 442.

[59] MOSSIGE, J.C., Amer. Natur. 89 (1955) 123.

[60] KVELLAND, I., Hereditas 53 (1965) 281.

[61] KVELLAND, I., Hereditas 54 (1965) 88.

[62] LEFEVRE, G. JR., JONSSON, U.B., Genetics 47 (1962) 1719.

[63] YANDERS, A.F., Genetics 49 (1964) 309.

[64] MOSSIGE, J.C., Repair from genetic damage. (SOBELS, F.H., Ed.) Pergamon Press, London (1963) 253.

[65] STRØMNÆS, Ø., KVELLAND, I., Genetics 50 (1964) 337.

[66] KVELLAND, I., STRØMNÆS, Ø., Hereditas 54 (1965) 170.

[67] LÜNING, K.G., J. Cell. Comp. Physiol. 58 (1961) 197.

[68] LEFEVRE, G. JR., JONSSON, U.-B., Mutation Res. 1 (1964) 231.

[69] LEFEVRE, G. JR., Mutation Res. 2 (1965) 22.

[70] WEDVIK, H., STRØMNÆS, Ø., Int. J. Rad. Biol. 7 (1963) 369.

[71] SOLLUNN, F.J., STRØMNÆS, Ø., Hereditas 51 (1964) 1.

[72] BONNIER, G., LÜNING, K.G., Hereditas 39 (1953) 193.

[73] ABRAHAMSON, S., TELFER, J.D., Genetics 39 (1954) 955.

[74] TRAUT, H., Repair from Genetic Radiation Damage (SOBELS, F.H., Ed.) (1963) 359.

[75] LEFEVRE, G. JR., Genetics 51 (1965) 381.

[76] OSTER, I.I., Radiation Biology 253 Proc. Sec. Australasian Conf. on Radiation Biology, University, Melbourne. Butterworths Scientific Publications, London (1958).

[77] LEFEVRE, G. JR., Genetics 53 (1966) 137.

[78] LEFEVRE, G. JR., Genetics 55 (1967) 263.

[79] Repair From Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells. (SOBELS, F.H., Ed.) Pergamon Press, London 1963.

[80] Genetical Aspects of Radiosensitivity: Mechanisms of Repair, Proceed. of a Panel Int. Atomic Energy Agency, Vienna (1966).

[81] von BORSTEL, R.C., "Effects of radiation on cells". Ch. 3, The Biological Basis of Radiation Therapy (SCHWARTZ, E.E., Ed.) Pitman Medical Publ. Co., LTD., London (1966).

[82] Radiation Research. Proc. Third Int. Cong. Rad. Res., Cortina d'Ampezzo, Italy, 1966. (SILINI, G., Ed.) North-Holland Publ. Co., Amsterdam (1967).

[83] SOBELS, F.H., Genetics Today, (GEERTS, S.J., Ed.) Proc. XI Int. Congress Genetics, Hague 1963 - Pergamon Press, London (1965) 235.

[84] SOBELS, H.F., in ref. [79] 179.

[85] SOBELS, F.H., MICHAEL, B., MUKHERJEE, R., OLIVIERI, G., SANKARANARAYANAN, K., WATSON, W.A.F., in refr. [82] 502.

[86] DICKERMAN, R.C., Genetics 48 (1963) 311.

[87] RINEHART, R.R., Genetics 49 (1964) 855.

[88] SAX, K., Proc. Nat. Acad. Sci. 25 (1939) 225.

[89] SAX, K., Cold Spring Harbor Symp. Quant. Biol. 9 (1941) 93.

[90] SAVAGE, J.R.K., in refr. [80].

[91] EVANS, H.J., in refr. [80] 31.

[92] MILLER, O.L. Jr., CARRIER, R.F., von BORSTEL, R.C., Nature 206 (1965) 905.

[93] von BORSTEL, R.C., MILLER, O.L., CARRIER, R.F., Chromosomes Today (DARLINGTON, C.D., LEWIS, K.R., Eds.) Oliver and Boyd, Edinburgh Vol 1 (1966) 141.

[94] KIRBY-SMITH, J.S., RANDOLPH, M.L., J. Cell. comp Physiol., Supplement 58 (1960) 1.

[95] CHU, E.H.Y., Mutation Res. 2 (1965) 75.

[96] REVELL, S.H., Radiation Induced Chromosome Aberrations. (WOLFF, S., Ed.) Columbia University Press, N.Y. (1963) 41.

[97] WOLFF, S., in refer, [80] 1.

[98] WOLFF, S., Amer. Nat. 94 (1960) 85.

[99] WOLFF, S., Mechanisms of the Dose Rate Effect of Radiation at the Genetic and Cellular Levels. Supplement J. Genetics 40 (1965) 38.

[100] STRØMNÆS, Ø., Hereditas (1968) In press.

[101] CALKINS, J., Int. J. Rad. Biol. 12 (1967) 297.

[102] SHIOMI, T., Mutation Res. 4 (1967) 323.

[103] SANKARANARAYANAN, K., Mutation Res. 4 (1967) 641.

DISCUSSION

R.C. VON BORSTEL: It would appear that meiosis proceeds more slowly in the radiation-sensitive strain. Do you believe that there may be a correlation between these two characteristics?

O. STRØMNAES: Yes, developmental rate may well be partially responsible for the difference in radiation sensitivity.

EFFETS COMPARES D'IRRADIATIONS AUX RAYONS GAMMA ET AUX NEUTRONS SUR LES ORGANES REPRODUCTEURS DE LA MOUCHE MEDITERRANEENNE DES FRUITS, Ceratitis capitata Wied. (DIPTERA: TRYPETIDAE)

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Abstract — Résumé

COMPARATIVE STUDY OF THE EFFECT OF GAMMA AND NEUTRON IRRADIATION ON THE REPRODUCTIVE ORGANS OF THE MEDITERRANEAN FRUIT FLY Ceratitis Capitata Wied. (DIPTERA:TRYPETIDAE). Plant radiobiology studies have demonstrated that plants grown from grains exposed to neutron irradiation show, for a given survival rate, greater sterility than those grown from grains exposed to gamma rays. It therefore seemed of interest to determine whether the same phenomenon occurs with insects, with a view to making use of it for pest control.

Treatment with gamma rays at sterilization doses (more than 3 krad) inhibits ovogenesis; at sub-sterilization doses a variable number of ovarioles may show normal development. In the male, at higher doses (8-10 krad), the spermatozoids formed remain normal and the testes retain their usual appearance, but spermatogenesis is greatly reduced.

Irradiation with neutrons by exposing pupae in a reactor core causes quite different effects. Relatively high doses of the order of 1000 rad inhibit the development of the gonads and leave the male with a residual fertility which is very weak or non-existent, the testes being atrophied by progressive reduction of the region containing spermatocytes and spermatogonia. In the females, the ovary appears as an undifferentiated mass. At lower doses, the females show a typical development of certain ovarioles, resulting in the formation of morphologically abnormal eggs; histological studies show an aberrant disposition of the vitelline cells, and various abnormalities.

EFFETS COMPARES D'IRRADIATIONS AUX RAYONS GAMMA ET AUX NEUTRONS SUR LES ORGANES REPRODUCTEURS DE LA MOUCHE MEDITERRANEENNE DES FRUITS Ceratitis capitata Wied. (DIPTERA: TRYPETIDAE). Des études de radiobiologie végétale ont montré que les plantes issues de graines irradiées aux neutrons présentent, à survie égale, une plus forte stérilité que celles provenant de graines ayant reçu des rayons gamma. Il paraissait donc intéressant de rechercher si le même phénomène se produit chez les insectes, en vue de son application à la lutte contre les ennemis des cultures.

Le traitement aux rayons gamma à des doses stérilisantes (plus de 3 krad) provoque chez la femelle un blocage de l'ovogénèse; aux doses substérilisantes un nombre variable d'ovarioles peut montrer un développement normal. Chez le mâle, à des doses plus élevées (8 à 10 krad) les spermatozoïdes formés restent normaux, mais la spermatogénèse est très fortement ralentie.

L'irradiation aux neutrons par exposition des pupes dans une pile a des effets assez différents. Des doses relativement fortes, de l'ordre de 1000 rad, induisent un blocage du développement des gonades et laissent chez le mâle une fertilité résiduelle très faible ou nulle: les testicules s'atrophient par réduction progressive de la zone à spermatocytes et à spermatogonies; chez la femelle l'ovaire apparaît comme une masse indifférenciée. Avec des doses plus faibles les femelles présentent un développement atypique de certaines ovarioles aboutissant à la formation d'œufs morphologiquement anormaux; l'étude histologique montre une disposition aberrante des cellules vitellines et diverses anomalies.

1. INTRODUCTION

L'efficacité biologique relative des neutrons sur des insectes a été mesurée par plusieurs auteurs. Les résultats, fondés soit sur des mesures de DL50, soit sur des mesures de taux de mutation, donnent des valeurs allant le plus souvent de 1,76 à 4 environ [1-6]. On doit donc s'attendre à ce que l'action des neutrons soit plus efficace que celle des rayons gamma pour obtenir la stérilisation d'insectes; ou encore que, pour obtenir le même taux de stérilité, il faille employer une dose moins forte d'irradiation aux neutrons qu'aux rayons gamma.

Nous savons par ailleurs que, chez les végétaux, l'action comparée des rayons et des neutrons montre, à taux de survie égal, une stérilité beaucoup plus élevée chez les plantes provenant de graines traitées aux neutrons [7-9]. L'action des neutrons sur les plantes est d'autre part assez régulière pour permettre la mise au point d'une technique de dosimétrie biologique; celle-ci est fondée sur la mesure de la première feuille des plantules issues de graines d'orge irradiées sèches [10-12]. Etant donné la difficulté de mesurer directement la dose d'irradiation en neutrons reçue dans un réacteur par du matériel biologique, il paraissait intéressant de comparer dans ces conditions l'action biologique sur les grains d'orge et sur un insecte, ici la mouche méditerranéenne des fruits Ceratitis capitata Wied.

Enfin, il nous paraissait également intéressant de comparer, sur C. capitata, l'action des rayons gamma, déjà partiellement étudiée [13], et des neutrons sur le développement et la structure des gonades. C'est d'ailleurs sur ce dernier point du programme en cours que les résultats les plus nets ont été jusqu'à présent enregistrés.

2. TECHNIQUES

Les pupes de C. capitata destinées à être irradiées proviennent d'un élevage permanent de la Station de zoologie de Montfavet.

Les irradiations sont pratiquées, pour les rayons gamma dans un irradiateur au cobalt-60 de 1200 Ci donnant un débit de dose de 1000 rad/min, et pour les neutrons dans un réacteur de type piscine, la pile PEGGY, de faible puissance (inférieure à 1 kW), le combustible étant de l'uranium enrichi à 90%. Ces installations se trouvent au Centre d'études nucléaires de Cadarache.

Les échantillons biologiques préalablement emballés dans des sachets de polyéthylène soudés sont plongés dans l'eau du réacteur, au contact de l'écran de protection du cœur (dans le premier centimètre d'eau le flux neutronique est de $5 \cdot 10^9$ n cm^{-2} s^{-1} quand la puissance du réacteur est de 300 W).

Les pupes ou les graines d'orge sont étalées sur des plaques pour que l'épaisseur de l'ensemble ne dépasse pas 1 cm. Dans ces conditions, la dosimétrie biologique avec l'orge a montré qu'une dose de 750 rad environ était atteinte en 12 min d'irradiation.

Les pupes irradiées sont ensuite mises en éclosion à la Station de zoologie de Montfavet. Les insectes éclos sont examinés quant à leur vitalité, leur fécondité et leur fertilité; leur dissection et l'examen histologique des gonades permet de comparer l'action des rayons gamma et des neutrons.

3. HISTOLOGIE DES ORGANES GENITAUX

3.1. Organes génitaux mâles

Les organes génitaux du mâle sont formés par deux testicules globuleux, de forme ovalaire, de 470 μm de longueur sur 180 μm de diamètre en moyenne.

Chez l'adulte mâle à l'émergence chaque glande génitale contient de nombreux cystes à spermatogonies et à spermatocytes ainsi que de nombreux faisceaux de spermatides et de spermatozoïdes.

Au cours des trois premiers jours de la vie imaginale la partie inférieure du testicule va se différencier progressivement en une zone contenant uniquement des spermatozoïdes individualisés. Cette sorte de poche représente alors la moitié environ de la taille de l'organe génital entier, ce dernier ayant atteint sa structure définitive.

3.2. Organes génitaux femelles

Les organes génitaux de la femelle à l'émergence sont représentés par deux ovaires méroïstiques polytrophiques de 270 μm de longueur environ, qui présentent la structure histologique suivante: d'une part le germarium constitué par la partie apicale des ovarioles contenant les ovogonies et les cellules folliculaires; d'autre part le vitellarium où s'effectuent la division et la différenciation des ovogonies en préovocytes et cellules vitellines pour former les follicules ou cytoblastes. A ce stade, chaque ovariole contient deux et quelquefois trois follicules à la suite les uns des autres et de taille croissante.

De 24 à 48 h après l'émergence on commence à noter une légère augmentation de volume des ovaires et à partir du deuxième jour la croissance se déclenche de façon très rapide. Chez les imagos de 3 j les ovaires atteignent une longueur de 650 μm en moyenne et contiennent des ovocytes à tous les stades de maturation, dont trois ou quatre en fin de développement. Au quatrième jour de vie imaginale ce nombre devient plus important et à partir du cinquième jour la maturité sexuelle des femelles est atteinte; pratiquement chaque ovariole contient un ovocyte complètement développé et prêt à être pondu au stade d'ovocyte I. A ce moment les ovaires occupent presque la totalité de la cavité abdominale des femelles avec une longueur de 800 à 1000 μm.

4. IRRADIATION DES PUPES AUX RAYONS GAMMA

4.1. Action sur la vitalité et la reproduction

L'action des rayons gamma sur C. capitata a déjà été étudiée par plusieurs auteurs sur le plan de la vitalité, la fécondité et la fertilité [13-15].

La durée de vie des insectes irradiés à 10 krad est souvent légèrement supérieure à celle des témoins.

L'irradiation des pupes de 8 j (à la veille de l'éclosion) jusqu'à la dose de 15 krad ne diminue pas le taux d'éclosion par rapport aux témoins.

L'irradiation des pupes à 1 krad abaisse la fertilité des adultes à 50% environ pour les femelles et 30% pour les mâles. L'irradiation

à plus de 3 krad provoque la stérilité totale des femelles par blocage du développement ovarien. Une dose de 10 krad est nécessaire pour obtenir une stérilité presque totale des mâles; dans ce cas les spermatozoïdes formés peuvent féconder l'œuf mais sont porteurs de mutations létales dominantes; la formation de nouveaux spermatozoïdes est bloquée.

4.2. Action sur les gonades mâles

L'étude histologique des organes génitaux mâles a été faite sur des individus âgés généralement de 4, 9 et 16 j après irradiation de pupes à 2, 3, 4, 5 et 9 krad.

Chez des individus âgés de 4 j les testicules conservent une taille normale quelle que soit la dose d'irradiation. La partie basale de ces organes présente un aspect normal et comprend une zone à spermatozoïdes individualisés ainsi que de très nombreux spermatodesmes. On ne voit plus de spermatides quelle que soit la dose. La partie apicale par contre est désorganisée. A 2 et 3 krad on peut encore apercevoir quelques cystes à spermatogonies; ces derniers disparaissent totalement aux doses supérieures. Ainsi, à 4 et 5 krad toute la partie apicale est formée par plusieurs amas de noyaux paraissant correspondre à des cystes dont les cellules auraient perdu leur membrane cytoplasmique.

Pour les individus plus âgés (7 à 9 j) la zone à spermatozoïdes individualisés est toujours bien développée et la différence porte surtout sur le nombre de spermatodesmes, qui est beaucoup plus réduit. Les spermatides sont toujours absentes. La partie apicale, particulièrement à la dose stérilisante de 9 krad, prend un aspect caractéristique présentant les amas trouvés à 4 j, mais ici arrondis, bien individualisés et remplis de noyaux ou tout autre corpuscule fortement coloré par l'hématoxyline. Chez les individus âgés de 16 j après irradiation à moins de 9 krad le testicule garde sa taille normale. Les spermatozoïdes occupent presque tout l'organe; on ne voit plus que quelques spermatodesmes. Après irradiation à 2 et 3 krad on aperçoit encore quelques cystes à spermatocytes, mais la zone apicale, comportant encore quelques amas cellulaires indifférenciés, a presque disparu. Après irradiation à 9 krad on ne voit plus de cystes et le testicule présente une taille réduite. Chez un mâle âgé de 1 mois après irradiation à 9 krad les testicules sont nettement atrophiés et réduits à l'état de deux sacs ne contenant que des spermatozoïdes individualisés.

Ces indications semblent signifier d'une part que, quelle que soit la dose, les spermatides se différencient rapidement en spermatozoïdes. D'autre part, aux doses faibles, la zone à spermatocytes apparaît partiellement désorganisée, partiellement bloquée dans son développement, mais capable encore pour une part d'une évolution normale. Enfin, à 9 krad il y a désorganisation puis dégénérescence de cette zone aboutissant à une atrophie de l'organe.

4.3. Action sur les gonades femelles

Contrairement aux gonades mâles les organes génitaux femelles sont très peu développés chez les pupes âgées de 8 j au moment de l'irradiation; ce fait pourrait permettre d'expliquer l'action beaucoup plus forte des rayons gamma à dose égale sur les femelles. Les coupes histologiques

de femelles irradiées à 2, 3, 4, 5 et 9 krad ont été effectuées sur les insectes âgés de 16 j. Déjà pour 2 krad les ovaires sont de taille réduite mais présentent une ovogénèse normale avec tous les stades de développement des follicules; cette diminution de taille provient surtout d'un nombre plus réduit d'ovocytes venus à maturité.

A la dose de 3 krad, au-delà de laquelle la stérilité est complète, nous trouvons une action beaucoup plus marquée et caractéristique des rayons gamma. Les ovaires, toujours pour les femelles de 16 j, sont de taille réduite mais peuvent présenter des structures variées. On peut trouver un certain nombre d'ovocytes normalement développés. On peut aussi en trouver un nombre très réduit, parfois même un seul. On constate par contre un nombre plus ou moins important de follicules atypiques: ovocytes plus ou moins identifiables, cellules nourricières irrégulières, parfois confondues en une masse indifférenciée, présence de nombreux petits noyaux ressemblant à ceux des cellules folliculaires et répartis au milieu des cellules nourricières.

A partir de 4 krad les deux ovaires de taille identique à celle qu'ils avaient au moment de l'irradiation ne subissent aucun développement. A la partie apicale les ovarioles contiennent un mélange formé par de petits follicules de structure aberrante, de nombreux noyaux de cellules folliculaires, des masses arrondies contenant de nombreux petits noyaux ainsi que des cellules vitellines isolées. La partie basale n'est formée que par les parois des ovarioles.

A 9 krad cette atrophie des ovaires est maximale et ces derniers ne sont plus formés que par les ovarioles vides dont la paroi cellulaire est plus épaisse dans la partie basale des gonades.

5. IRRADIATION DES PUPES AUX NEUTRONS

5.1. Action sur la vitalité et la reproduction

Les insectes ont été irradiés aux neutrons au stade pupe âgée de 8 j, par exposition plus ou moins longue au flux de la pile PEGGY, généralement pendant des temps de 5, 10, 15 et 20 min.

Les taux d'émergence de ces pupes irradiées sont absolument identiques à ceux observés chez les insectes témoins et dépassent dans tous les cas 95%. (Dans la détermination de ce taux d'émergence ne sont pris en considération que les imagos entièrement débarrassés de leur puparium et non ceux qui se trouvent à moitié ou presque sortis de leur enveloppe nymphale, bien qu'ils puissent rester ainsi vivants pendant quelque temps.)

La longévité des adultes a été contrôlée dans toutes les expériences pendant une durée de 20 j après leur émergence. Des temps d'irradiation de 5 à 15 min ne semblent pas affecter la longévité des insectes en comparaison de celle d'insectes normaux, tout au moins jusqu'au vingtième jour, où la mortalité est en moyenne de 10%. Seuls des temps d'irradiation de 20 min diminuent légèrement la longévité des femelles et plus fortement celle des mâles (35% de mortalité pour ces derniers) (fig. 1).

En ce qui concerne l'étude de la fécondité et de la fertilité, les résultats obtenus sont assez variables; ceci est peut-être dû au fait que les doses reçues pendant un même temps d'exposition sont elles-mêmes variables d'une séance d'irradiation à l'autre. Cependant les effets de ces irradia-

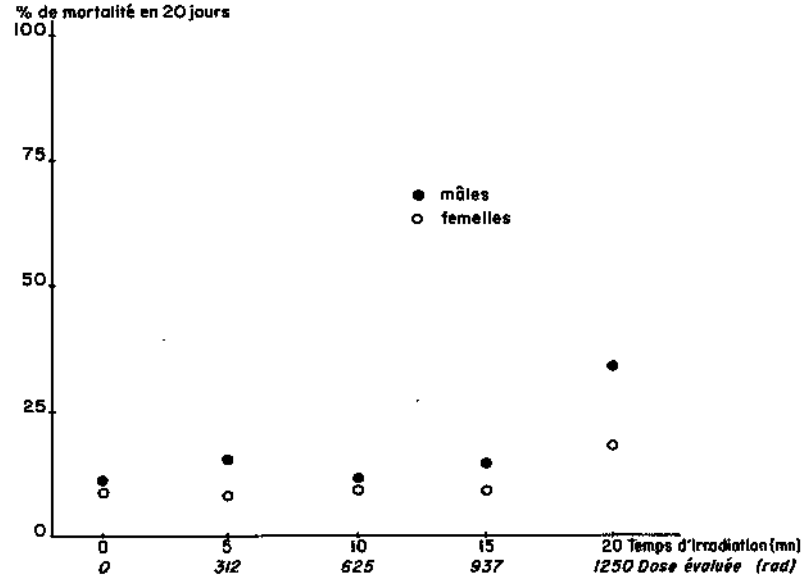

FIG. 1. Pourcentage de mortalité totale pour des lots d'insectes (1000 individus environ pour chaque lot) exposés aux neutrons pendant des temps variés.

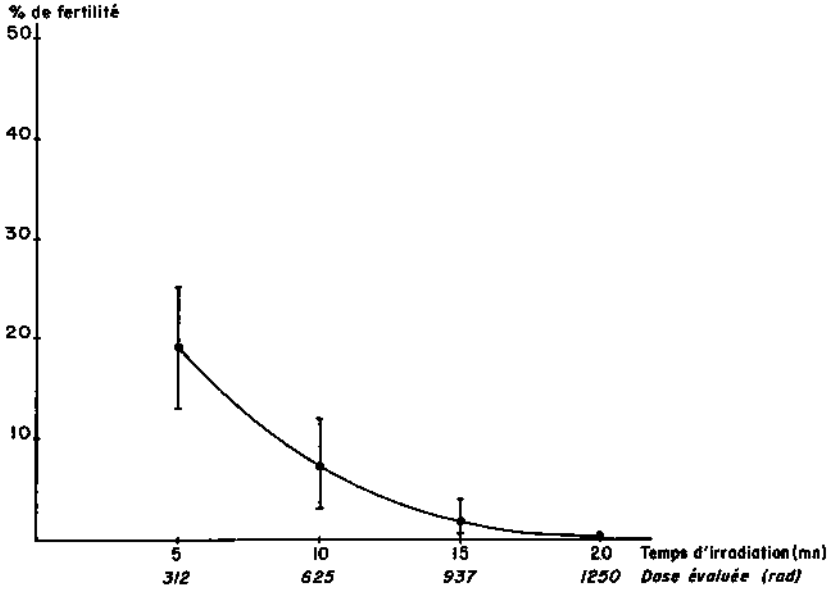

FIG. 2. Fertilité résultant de croisements ♀ normales × ♂ issus de pupes de 8 j irradiées aux neutrons.

tions sont déjà très sensibles pour des temps d'exposition de 2 à 5 min et augmentent avec des temps plus élevés.

En ce qui concerne les mâles, des croisements effectués entre mâles irradiés et femelles normales donnent une fertilité moyenne de l'ordre de 20% pour des expositions de 5 min, et de 7 à 8% pour des expositions de 10 min. Les doses stérilisantes ne sont atteintes qu'à partir de 15 min, où subsiste encore une fertilité résiduelle de 1,7%, cette fertilité n'étant plus que de 0,3% à 20 min (95% étant la fertilité moyenne chez les témoins) (fig. 2).

Mais le fait le plus intéressant est la différence d'action qui semble exister entre les irradiations aux rayons gamma et aux neutrons chez les femelles.

Rappelons que les doses substérilisantes de rayons gamma permettent la formation d'un nombre réduit d'œufs, mais que ceux-ci sont morphologiquement normaux. Dans le cas de nos expositions aux neutrons nous avons obtenu une stérilité totale des femelles à partir de 15 min d'exposition. Par contre, pour 10 min d'exposition nous avons observé la formation et la ponte d'œufs morphologiquement anormaux; ceux-ci apparaissent déformés, parfois raccourcis jusqu'à devenir globuleux, et présentent souvent une structure interne hétérogène visible à travers le chorion.

5.2. Action sur les gonades mâles

Toutes les observations portant sur des insectes issus de pupes irradiées aux neutrons pendant des temps de 5, 10, 15 et 20 min ont été effectuées sur des individus âgés de 25 j.

Chez les mâles irradiés pendant 5 min, dose qui diminue considérablement la fertilité, nous ne constatons aucune modification au niveau des gonades, qui restent de taille et de structure analogues à celles d'organes génitaux de mâles normaux.

L'action est beaucoup plus nette et apparaît de façon brutale chez des insectes irradiés pendant 10 min. En effet, chez ces individus les testicules peuvent être tout deux très atrophiés et réduits à des sortes de poches allongées ne contenant que des spermatozoïdes individualisés avec parfois quelques cystes à spermatogonies. Mais, dans le cas le plus général, ces mâles ne présentent qu'un seul des organes génitaux ainsi atrophié, alors que l'autre conserve une structure normale présentant tous les stades de la spermatogénèse, sauf peut-être quelques modifications aux niveaux des spermatogonies et spermatocytes.

Pour des temps d'irradiation de 15 et 20 min il y a toujours blocage complet de la spermatogénèse, et les organes génitaux sont tous deux réduits à des sortes de sacs à parois plus épaisses ne contenant que des spermatozoïdes avec quelques rares spermatodesmes à 15 min.

5.3. Action sur les gonades femelles

Comme dans le cas des rayons gamma l'irradiation aux neutrons, même à faible dose, provoque d'importantes modifications au niveau des cellules reproductrices.

Déjà pour des temps d'irradiation de 5 min les organes génitaux sont à la fois de taille réduite (de moitié environ par rapport à la normale) et l'ovogénèse est plus ou moins perturbée. Parmi les follicules se

développant normalement se trouvent quelques follicules atypiques ayant l'aspect de masses arrondies de différentes formes comportant, soit en totalité, soit simplement en bordure, une multitude de petits noyaux. Les différences individuelles sont très importantes d'une femelle à l'autre; dans certains cas l'ovaire ne contient qu'un ou deux follicules aberrants, dans d'autres au contraire il est entièrement formé par ces masses arrondies où se trouvent encore quelques follicules normaux.

A la suite d'une irradiation de 10 min, les gonades sont généralement de taille encore plus réduite, mais les différences individuelles subsistent toujours. On peut noter dans tous les cas un nombre plus ou moins important de ces masses tissulaires amorphes remplies de petits noyaux, qui paraissent être caractéristiques des irradiations aux neutrons. Ces « follicules » atypiques de formes irrégulières, qui peuvent même fusionner entre eux, dénotent une certaine prolifération cellulaire. Sur certaines coupes histologiques on peut noter l'analogie existant entre ces noyaux de prolifération cellulaire et ceux des cellules folliculaires qui subsistent en bordure par endroits.

Aux doses stérilisantes après irradiation de 15 ou 20 min, les résultats sont plus homogènes et nous observons une action comparable à celle produite par les rayons gamma aux doses supérieures à 4 krad. L'ovogénèse est inhibée par blocage des divisions cellulaires, et les ovaires entièrement atrophiés, de taille comparable à ceux de femelles immatures, ne contiennent aucun follicule (du moins en ce qui concerne des individus femelles âgés de 25 j). Ces gonades ne sont formées que par une série d'ovarioles vides plus fortement colorées à leur partie basale, comme nous avions vu dans le cas d'irradiations aux rayons gamma à partir de 4 krad.

6. CONCLUSION

La comparaison de l'action des rayons gamma et des neutrons sur C. capitata a été faite en partant de l'hypothèse d'une efficacité biologique relative des neutrons supérieure à celle des rayons gamma. La difficulté de mesurer directement la dose d'irradiation aux neutrons dans le réacteur nous a fait utiliser un étalon biologique connu, la croissance de plantules issues de graines d'orge irradiées. Dans ces conditions, des temps d'exposition aux neutrons de 5, 10, 15 et 20 minutes correspondaient à des doses calculées de 310, 620, 940 et 1250 rad.

Les irradiations de pupes âgées jusqu'aux doses stérilisantes de rayons gamma ou de neutrons ne diminuent pas les taux d'éclosion. Par contre la longévité des adultes, non affectée par les rayons gamma jusqu'à 10 krad, est diminuée après irradiation aux neutrons à 1250 rad.

L'efficacité biologique relative élevée des neutrons apparaît lorsqu'on examine les taux de fécondité et de fertilité. Dans les conditions de nos expériences, on peut en déduire que l'efficacité biologique des neutrons a été environ de 7 fois celle des rayons gamma.

L'action des rayons gamma et des neutrons sur les gonades mâles paraît assez semblable. Aux doses faibles les testicules restent de dimensions normales, mais l'examen histologique montre que la spermatogénèse a été arrêtée. Aux doses élevées, dites stérilisantes, on constate que les gonades sont réduites à des sortes de sacs contenant uniquement les spermatozoïdes.

L'action des rayons gamma et des neutrons sur les gonades femelles aux doses stérilisantes provoque l'atrophie complète des ovaires.

Par contre, aux doses substérilisantes apparaît une différence d'action d'autant plus marquée que l'on approche de la dose stérilisante. Après irradiation aux rayons gamma on constate la ponte d'un nombre réduit d'œufs, ceux-ci présentant un aspect normal. Après irradiation aux neutrons on constate aussi la ponte d'un nombre réduit d'œufs mais une partie de ceux-ci ont un aspect anormal, déformé, souvent globuleux; ces œufs sont stériles. L'examen histologique confirme dans les ovaires la présence de follicules aberrants constitués de masses tissulaires amorphes.

Il apparaît donc que l'efficacité biologique relative des neutrons est supérieure à celle des rayons gamma, mais qu'en même temps le mode d'action des neutrons sur les organes reproducteurs n'est pas exactement semblable à celui des rayons gamma.

REFERENCES

[1] ABELEVA, E.A., LAPKIN, YU.A., Radiobiologiya 2 2 (1962) 293-97.
[2] ABELEVA, E.A., LAPKIN, YU. A., Radiobiologiya 3 3 (1963) 420-21.
[3] BICKER, A.E., Thesis, Kansas Univ., Lawrence, TID-15962 (1962) 52 p.
[4] MURAKAMI, A., KONDO, S., Rep. natn. Inst. Genet., Misima 14 (1963) 106-07.
[5] MURAKAMI, A., KONDO, S., Jap. J. Genet. (Idengaku Zasshi) 39 (1964) 102-14.
[6] MURAKAMI, A., KONDO, S., TAZIMA, Y., Jap. J. Genet. (Idengaku Zasshi) 40 (1965) 113-124.
[7] ERENBERG, L., SEALAND, E., J. nucl. Energy 1 (1964) 150-69.
[8] MAC-KEY, J., Hereditas 37 (1951) 421-64.
[9] PEREAU-LEROY, P., Bull. Inf. Sci. Tech. C.E.A. 93 (1965) 19-26.
[10] AHNSTRON, G., GUERRIERI, G., GIACONELLI, M., DI PALO, L., Lab. Applic. Agric. C.S.N. Casaccia, Public. n°86 (1964).
[11] ARNAUD, Y., Bull. Inf. Sci. Tech. C.E.A. 82 (1964) 58-62.
[12] KONZAC, C.F., Radiat. Res. 1 (1954) 220.
[13] FERON, M., Annls Epiphyt. 17 2 (1966) 229-39.
[14] ARROYO, M., JIMENEZ, A., MELLADO, L., CABALLERO, F., Boln. Patol. veg. Ent. agric. 28 (1965) 257-98.
[15] KATIYAR, K.P., VALERIO, J., Turrialba 14 (1964) 211-21.

DISCUSSION

W.F. BALDWIN: It is usually impossible to obtain gamma-free neutron irradiations from reactors. Did this present any problem for you?

P. PELEGRIN: Perhaps I might answer this on Dr. Causse's behalf. The neutron radiation came from the PEGGY swimming-pool reactor at Cadarache, in the tank of which fast neutrons are generated at the same time as gamma rays and thermal neutrons. The neutron spectrum is not clearly known, but it is known that the gamma flux is low. A worker in France wishing to investigate further could use the 'biological cavity' of the EL3 reactor at Saclay where, by means of a uranium converter, a high flux of fast neutrons is made available with almost total absence of gamma rays.

W.F. BALDWIN: In the one or two studies available for comparison it has been shown that fast neutrons (14 MeV) give an increase in effect over that of gamma radiation of about 1.4 (or approximately 40%), whereas

in your work you found a seven-fold increase. I should be interested to learn of the method whereby you compared the neutron and gamma effects.

P. PELEGRIN: As stated in the paper, the relative biological effectiveness of the neutrons, as compared with that of the gamma radiation, has been determined by Dr. P. Pereau-Leroy, at both Saclay and Cadarache, with dry irradiated barley grains, and his figures are comparable to those given here.

W.J. LE QUESNE: Would it be possible to make use of neutron sources of the type consisting of a mixture of an alpha-emitter and beryllium in this work, and would this make for a lower gamma dosage than that obtained using a reactor? I understand that emissions of up to 10^8 neutrons/s can be obtained, and wonder whether this level would be sufficiently high.

R. CAUSSE: I think it is quite feasible to use other neutron sources in order to cut out the gamma radiation. One could even eliminate it completely by a careful choice of isotope, but in this case it would certainly not be possible to obtain such a high neutron flux.

INCREASED YIELD OF GAMMA-INDUCED EYE COLOUR MUTATIONS FROM CHRONIC VERSUS ACUTE EXPOSURES IN Dahlbominus

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Abstract

INCREASED YIELD OF GAMMA-INDUCED EYE COLOUR MUTATIONS FROM CHRONIC VERSUS ACUTE EXPOSURES IN Dahlbominus. The interpretation of apparent differences in the mutagenic effects of chronic and acute irradiations is often complicated because the exposed cells may not have remained in comparable stages of the mitotic cycle in the two circumstances and because it is usually impossible to test for differential killing following the two treatments. Thus, in the first case, the early part of a chronic exposure could have the effect of slowing down the rate at which cells proceed through the subsequent normal sequence of changes in radiosensitivity. A method of avoiding this source of bias in studies of the eye colour mutations of Dahlbominus was sought, and it has been found that susceptibility to induced mutation reaches a plateau in aging females about nine days after they become adult, and that over the following four days the mutation rate maintains a comparatively constant frequency. Accurate records of the number of cocoons parasitized by Dahlbominus and the number of progeny per female have also made it possible to test for differential killing by ascertaining whether decreased fecundity is associated with a depression of mutagenic rates.

Studies of the effects of 500 R of gamma radiation delivered chronically over the period of comparable sensitivity, i.e. over 100 hours from 9 days to 13 days after the pupal stage, and of the same dose as acute exposures at different times in this period are now complete, and are complemented by similar studies with 250 R. The results with 500 R indicate an approximately 20% greater mutagenic effect of the chronic as compared with the acute exposures. At the present time, an interpretation in terms of a possibly greater lethal effect of the acute irradiations on cells that are potentially more susceptible to mutagenesis cannot be ruled out. However, any contribution from this source should diminish with lower, less lethal doses. The results at 250 R show that the difference in mutation frequency with acute as compared with chronic doses is less at this lower exposure, implying that the difference in effect is probably the result of a disproportionately greater killing by the acute irradiation of cells which would have produced mutations. Records of parasitization and the number of progeny per female following acute and chronic exposures at 500 and 250 R give conflicting results, indicating that differential killing may not be a factor in determining mutagenesis. The results are based on 923 eye colour mutations in a total of 346 171 flies scored.

1. INTRODUCTION

Early work with Dahlbominus on the effects of acute and chronic dose rates failed to demonstrate differences in the frequencies of induced eye colour mutations in irradiated oögonia exposed to similar total doses [1]. A further extension of the studies, however, did indicate a small but statistically significant fall in mutation rate with chronic, as compared with acute, exposures of oögonia [2], an effect which supported results with other organisms such as mice [3] and silkworms [4], where mutation rates were likewise higher with acute doses. The effect was most apparent in mice, and Russell was able to show in spermatogonia that

exposure rates of 0.009 R/min gave only one-quarter as many specific locus mutations as compared with a rate of 90 R/min. These results, and those from other, similar studies, have led Russell to postulate that some step in the radiation-induced mutation process may be capable of repair, a phenomenon which would have important implications in the estimation of genetic hazards in man. In reaching his conclusions, Russell used data from female mice, which showed a much greater dose-rate effect than had been observed for spermatogonia. In female mice, germ cells remain in the dictyate stage of the primary oöcyte from shortly after birth until ovulation; thus, the dose-rate effect in females occurred in a non-dividing cell population [5]. Russell also attaches some significance to the fact that oöcytes in late follicle stages are resistant to killing by radiation, a condition which would lessen the possibility of a mutation rate difference as a result of differential cell survival following acute and chronic irradiations.

In the Dahlbominus work mentioned above [2], in which a significant difference in mutation was found with the two different treatments, females irradiated as larvae were allowed to deposit eggs on host cocoons from the age of four days until death; the progeny thus included individuals from all stages of oögenesis. As shown earlier [6], mutation rates in females increase sharply with age, and in adults irradiated at different ages the number of mutants at four days is approximately five times greater than in pupae, presumably the result of an increase in the number of highly sensitive oöcytes. In the present experiments, it was discovered that Dahlbominus females irradiated at 9, 11, and 13 days, when only mature oöcytes were present in the ovarioles, gave comparable mutation frequencies. Thus, chronic exposures could be administered over this period for comparison with acute doses. Under these conditions, the frequency of mutation was found to be lower with acute exposures than with chronic, a result which was the reverse of the effect shown by Russell in mice.

2. MATERIALS AND METHODS

An advantage of Dahlbominus as a subject for genetical studies is that all mutations induced in female unfertilized germ plasm will appear in first generation parthenogenetic haploid males. In addition, the scoring of eye colour mutants in this species is comparatively easy, the colours appearing as mutant phenotypes russet (ru), chestnut (cs), carmine (c) and claret (ct), contrasting sharply with the almost black wild type eye colour. As described recently, these phenotypes can arise at a minimum of 8 loci, demonstrating two linkage groups and two allelic series of three alleles each [7]. A description of the biology of Dahlbominus and the different eye colours has been published elsewhere [8].

Irradiation of experimental females was done daily in small plastic containers at about 200 females per day. Treatments at both acute and chronic rates were continued until sufficient unmated females had been placed with cocoons to produce at least 35 000 males. The acute exposures were done with a Gammabeam 150 (Commercial Products, AECL, Ottawa) containing approximately 700 Ci of ^{60}Co; for chronic irradiations, lasting over four days at each exposure, a smaller source of about 100 mCi of

^{60}Co was placed in a chamber where temperature and humidity could be maintained at rearing room levels. The insects were positioned at appropriate distances to give the lowest possible chronic exposure for a four-day period. The dose rates for the 500 and 250 R total exposures in these tests were given at an acute rate of 100 R/min, while the chronic dose rates amounted to 0.082 and 0.041 R/min, respectively. The total doses and dose rates were measured by means of a calibrated E. I. L. portable electrometer (Model 37A).

3. OBSERVATIONS

3.1. Mutation frequencies in broods of advancing ages

Since prior results had shown that age was a determining factor in the increase in mutation sensitivity of Dahlbominus, the first experiment of this series was designed to compare the mutation frequency in eggs deposited by unmated females at one, two, three and four days after irradiation. Daily exposures of successive groups of unmated females at four days of age were continued (exposure 1000 R at 100 R/min) until a sufficient number to produce at least 3500 males had been placed with host cocoons. The cocoons in each group were replaced each day over a four-day period, producing separate broods for each of the four days (Fig. 1). The mutation frequency, originally at 600 ($\times 10^{-5}$) on the first day, fell rapidly to a level at four days, approximating the frequencies shown previously for oögonia (50×10^{-5}) (Baldwin [1]). This finding proved that variable sensitivity in various stages of oögenesis would constitute a source of significant error in mutation frequency tests, especially if adult females were left with cocoons for several days after irradiation. Thus, in this work, all females were removed from cocoons after 24 hours. The same factor would be important with chronic treatments extending over several days, especially if the early part of the exposure slowed development, and a study of egg production in unmated females were undertaken to determine when irradiations could be confined to a single stage for both the acute and chronic treatments.

3.2. Oögenesis in ovarioles

Unmated female insects were dissected at the ages of 60, 132 and 252 hours after emergence from the pupal stage. The reproductive systems were teased away from surrounding abdominal tissue and displayed on a slide in a small drop of water. In Fig. 2(a) it can be seen that each ovary is composed of seven ovarioles each emptying into a common oviduct. Normally, the eggs would be fertilized after leaving the oviduct. In these experiments, however, all males were discarded as pupae making it impossible for fertilization to occur. At 60 hours of age (Fig. 2 (b)) the germarium contained numerous oögonia, while immature oöcytes (with nurse cells) and mature oöcytes, ready to be discharged, were present in the ovariole. In the ovarioles from 132-hour females (Fig. 3 (a)), all traces of oögonia had disappeared, and only immature and mature oöcytes were visible. In the last illustration (Fig. 3 (b)), at 252 hours, only mature oöcytes were present, a result which indicated that females

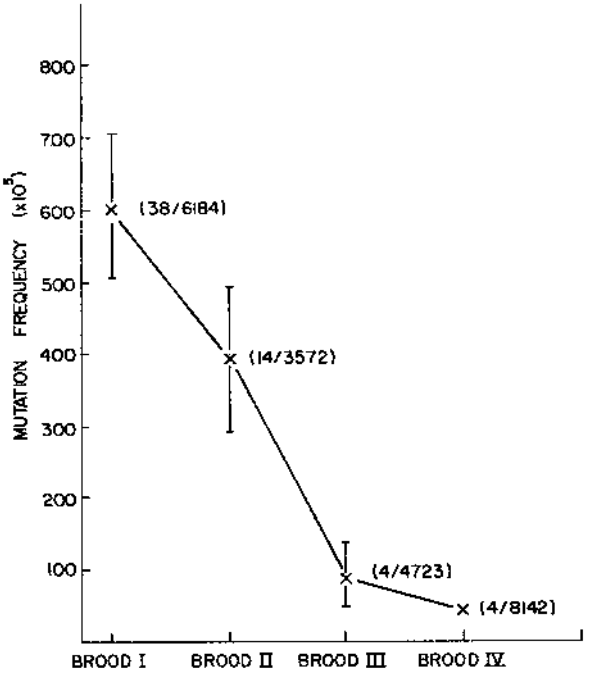

FIG. 1. Decreasing mutagenesis with increasing age in irradiated Dahlbominus females.

irradiated at this age would produce only mature oöcytes, at least for the first 24 hours. In these older flies, disintegrating oöcytes (see Fig. 3(b)) were found in many individuals.

From these dissections, it appeared that the production of new eggs ceased five days after emergence; by ten days, all evidence of oögonia and immature oöcytes had disappeared, proving that only mature oöcytes would be present for irradiation at these ages.

To substantiate these findings further, counts of mature oöcytes in the ovaries were made over the period from emergence to 20 days of age. In Figure 4, the average numbers of mature oöcytes from eight females at different ages show that the totals rose to a plateau at five days; from here the numbers present remained constant to about ten days of age, where a gradual decrease began, the result of the re-absorption of older oöcytes. Thus, it was concluded that the number of highly mutable mature oöcytes remains constant over a considerable period, and that it would be possible to administer chronic doses during a period when sensitivity to mutation would remain comparatively constant.

3.3. Mutation frequencies at different ages

To compare the mutation frequencies over the period when comparable numbers of oögonia were found in the ovarioles, females were irradiated (500 R at 100 R/min) at 7, 9, 11 and 13 days after emergence. The results, in Table I, show that the frequencies at these ages were, in fact,

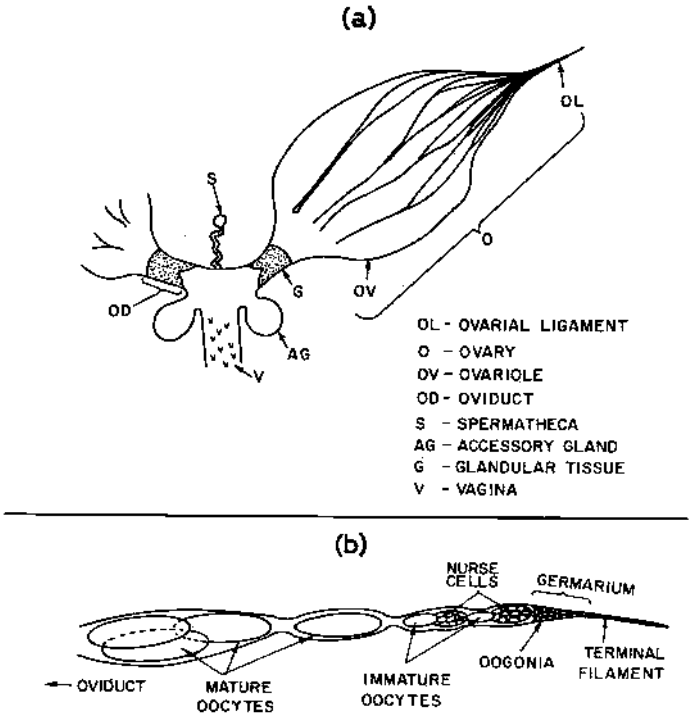

FIG. 2. (a) Schematic drawing of ovary of Dahlbominus female, showing 7 ovarioles on each side. (b) Ovariole at 60 hours of age, illustrating presence of oögonia, immature oöcytes, and mature oöcytes.

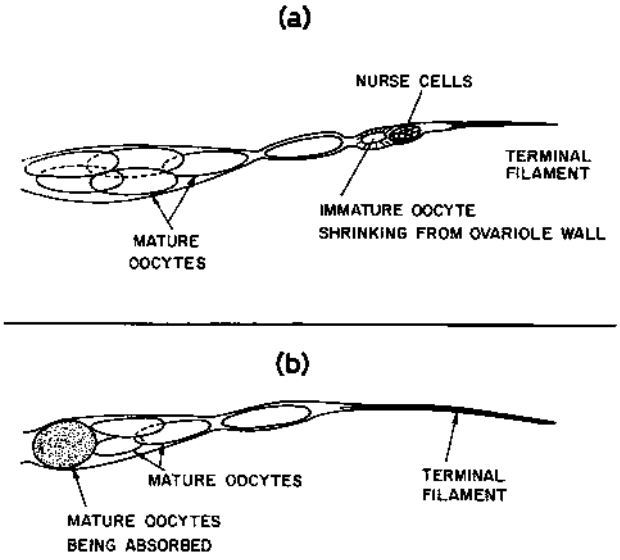

FIG. 3. (a) Ovariole at 132 hours, showing absence of oögonial cells. (b) Ovariole at 252 hours (10 days) showing absence of all stages except mature oöcytes.

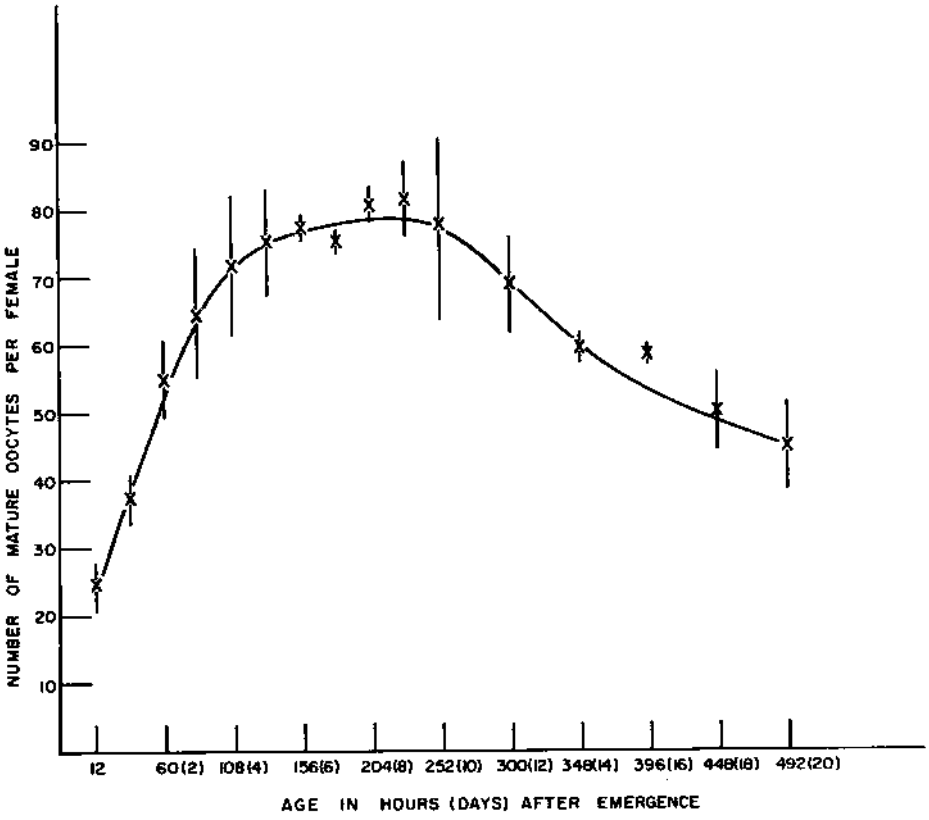

FIG. 4. Increasing numbers of mature oöcytes in aging females with plateau at 6 to 10 days.

TABLE I. MUTATION FREQUENCIES OVER THE PERIOD FROM 7 TO 13 DAYS AFTER EMERGENCE OF ADULT FEMALES (500 R at 100 R/min).

Age (days)	Offspring	Mutants	Mean frequency ($\times 10^5$)
7	57 984	160	276 (311 - 240)
9	46 831	140	298 (340 - 258)
11	35 136	129	367 (426 - 307)
13	49 987	176	352 (374 - 310)
Control	42 048	8	19 (29 - 8)

almost comparable in magnitude. The level at 11 days was higher than the 13-day value owing to a cluster of seven claret eye colour mutants in this group; when the cluster was considered as one mutation, the calculated frequency was exactly equal to the value at 13 days (350×10^{-5}).

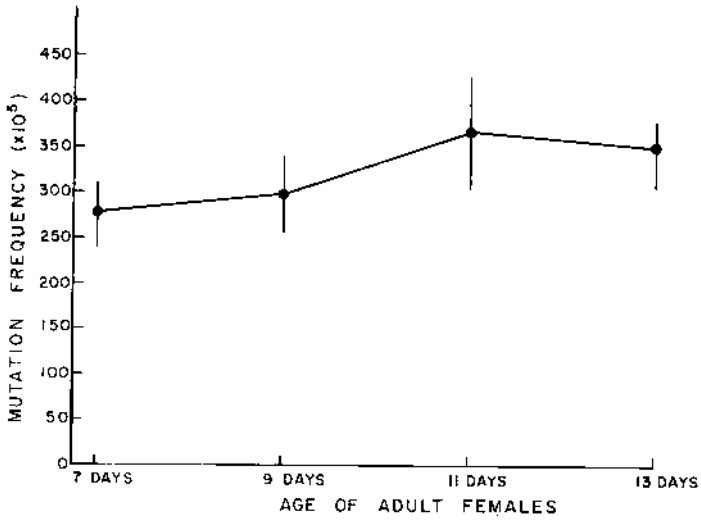

FIG. 5. Mutation frequencies following irradiation of females of different ages at 500 R (100 R/min).

The plateau from 9 to 13 days (Fig. 5) constituted a four-day period when chronic exposures could be administered for comparison with combined values from the acute irradiations at 9, 11 and 13 days.

3.4. Mutation frequencies with acute and chronic dose rates

Experiments to compare the effects of acute and chronic radiation were completed at two dose levels, 500 R and 250 R. At the higher dose, the acute value was derived by combining the data from 9, 11 and 13 days shown in Table I and Fig. 5. At 250 R, females at 11 days of age were used for acute irradiations. Chronic exposures for both doses extended from 9 to 13 days after emergence, at 0.08 R/min for the 500 R dose and at 0.04 for the 250 R exposure.

As shown in Table II, the mutation frequencies for the chronic irradiations are higher at both dose levels than in the acute exposures. At 500 R, the difference is significant ($P < 0.002$); at the 250 R exposure the limits overlap. In Fig. 6, an almost exact linear relationship exists between the values at 0, 250 and 500 R; also, the difference in effect at the two dose levels of chronic irradiation is apparent in the figure.

3.5. Fecundity of females following acute and chronic exposures

In these studies with Dahlbominus, a test for bias involving differential killing of potentially mutant eggs is available from rearing data. In the dose rate experiments, accurate records of the number of cocoons parasitized by the females and the number of haploid male progeny produced by the females were maintained. The results (Table III) show first that the percentage of females parasitizing cocoons and the number of viable eggs per female were both reduced in the irradiated groups.

TABLE II. DATA FROM TESTS OF ACUTE AND CHRONIC IRRADIATIONS AT 500 AND 250 R

Exposure (R)	R/min	Offspring	Mutants	Mean frequency (x 10^5)	Clusters
500	100	131 954	455	377 (363 - 311)	4 (7,2,2.2)[a]
500	0.08	62 966	270	428 (471 - 385)	1 (2)
250	100	57 780	99	171 (199 - 143)	2 (2,4)
250	0.04	51 423	101	196 (277 - 165)	1 (2)
Control	-	42 048	8	19 (29 - 8)	-

[a] Eye colour of all individuals in all clusters was claret

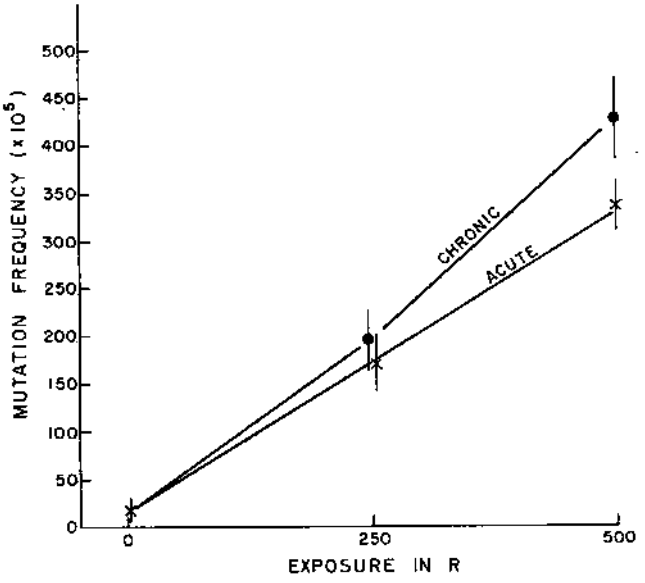

FIG. 6. Mutation frequencies following acute and chronic irradiations at 500, 250 and 0 R.

At 500 R, the chronic irradiation gave less parasitization and fewer offspring per female, while at 250 R, these two figures were reversed, with chronic exposures giving slightly more in both cases. Thus, it does not seem that differential killing in the egg stage could possibly be important in determining the relative mutation frequencies following acute and chronic exposures.

TABLE III. NUMBER OF PARASITIZED HOST COCOONS AND NUMBER OF PROGENY PER FEMALE FOLLOWING ACUTE AND CHRONIC EXPOSURES AT 500 AND 250 R

Exposure	Exposure rate	Number of cocoons	Number parasitized	%	P	Number of females	Number of males	Mean number of males	P
500	Acute (a)	2532	2038 (b)	80.4		2038	35136	17.4	
500	Chronic	8225	4966	60.3	< 0.001	4966	62966	12.3	< 0.001
250	Acute	5167	3016	58.3		3016	57780	16.1	
250	Chronic	4321	2823	65.3	< 0.001	2823	51423	18.6	< 0.001
Control	-	1305	1100	84.2	< 0.001	1100	42048	38.2	< 0.001

(a) Data from 11 days of age only used in this table.
(b) Not significantly different from controls ($P > 0.05$).

4. DISCUSSION

The most important fact to emerge from this research was that mutation frequencies following acute exposures were not so high as those from chronic doses in mature oöcytes at one stage of development and at two different dose levels. The experimental conditions in Dahlbominus were unique, since adult females were held for irradiation until 9 to 13 days, when only mature oöcytes were present in the ovarioles. Thus acute and chronic doses could be administered over a period of four days while the germ cells remained in the same stage, a situation which has not been achieved in other organisms, with the possible exception of dictyate oöcytes in the mouse. In Drosophila, Purdom [9] states that the various stages of spermatogenesis vary considerably in their sensitivity to irradiation, and strictly comparable sampling from successive stages of the development of the fly is impossible when different periods are used. He does conclude, however, that a dose rate effect comparable to that in the mouse does not occur in the more mature spermatogonial cells of Drosophila. Tazima [10] believes that two types of dose-rate dependence occur in young gonial stages of the silkworm, one consisting of a lower mutagenic effectiveness in chronic rather than in acute irradiation, and another in which chronic effects are higher than for acute treatments. Here again, however, this author stresses the complexity in interpreting dose rate data due to developmental stage. In Drosophila, Oster [11] states that despite the large-scale work already carried out, there is still no clear-cut basis for a dose rate effect in the production of point changes in immature female cells, and the work has been complicated by "differential radiosensitivity masking repair mechanisms". Thus, Russell's results showing significant mutagenic differences with dose rate, and his conclusions concerning premutational repair in mouse cells stand alone, seemingly unsupported by concrete evidence from other organisms.

In considering the responses to mutation at the two dose levels in Dahlbominus, it was interesting that the difference between the acute and chronic treatments was greater at 500 than at 250 R. As the lower dose can be presumed to be less lethal than the higher one, this result would be expected if the acute irradiation selectively killed cells which would have produced eye colour mutations. The parasitization and progeny records reveal, however, that more cocoons were parasitized and more progeny were produced following acute rather than chronic exposures at 500 R, and that these results were reversed at 250 R. Thus, it seems impossible that selective killing or selective survival of potentially mutable cells could account for the smaller difference in mutation rate at a lower, less lethal dose. Further, the number of progeny from irradiated females in this work was less than half the number produced in controls. Thus, extensive killing occurred in mature oöcytes, an effect which did not seem to produce differential mortality in potentially mutable cells.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. H.B. Newcombe for his continuing interest in the work, to

Messrs P. and A. Knight for their excellent technical assistance, and to Miss R. Tanner, who, as a summer student, studied the reproductive processes in Dahlbominus reported here.

REFERENCES

[1] BALDWIN, W.F., Radiat. Res. 27 (1962) 127.
[2] BALDWIN, W.F., Mutation Res. 2 (1965) 55.
[3] RUSSELL, W.L. et al, Science 128 (1958) 1546.
[4] TAZIMA, Y.S. et al, Genetics 46 (1961) 1335.
[5] RUSSELL, W.L., Proc. Conf. on Mech. of the Dose Rate Effect of Radiation, Oiso, Nov. 4-7 1964, 128.
[6] BALDWIN, W.F., Mutation Res. 2 (1965) 530.
[7] BALDWIN, W.F. et al, Can. J. Genet. Cytol. 2 (1966) 245.
[8] BALDWIN, W.F., et al, Can. J. Genet. Cytol. 4 (1964) 453.
[9] PURDOM, C.E., McSHEEHY, T.W., Int. J. Radiat. Biol. 7 (1963) 265.
[10] TAZIMA, Y., Proc. Conf. on Mech. of the Dose Rate Effect of Radiation, Oiso, Nov.2-7 1964, 68.
[11] OSTER, I.I., Proc. Conf. on Mech. of the Dose Rate Effect of Radiation, Oiso, Nov.2-7 1964, 83.

DISCUSSION

R.C. VON BORSTEL: I should like to point out that Dr. Baldwin is one of the few investigators who is in the process of providing mutation data where mutation frequencies can actually be analysed at frequencies below the spontaneous mutation level. By plotting the data on a log-log plot of mutation frequency versus dose, and by subtracting the spontaneous mutation frequencies from the induced mutation frequencies, it can be determined whether linearity is maintained, even at the very low doses where the spontaneous mutation data and the induced mutation data appear to merge.

REPRODUCTIVE PERFORMANCE OF FEMALE BRACONIDS COMPARED AFTER (A) BRIEF AND (B) PROTRACTED EXPOSURES TO IONIZING RADIATIONS

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Abstract

REPRODUCTIVE PERFORMANCE OF FEMALE BRACONIDS COMPARED AFTER (A) BRIEF AND (B) PROTRACTED EXPOSURES TO IONIZING RADIATIONS. ^{85}Sr sources are installed in the initial US biosatellites to provide calculable dose levels during three-day orbital space flights. Such protracted exposures are longer than those used customarily in insect radiobiology and shorter than those of ecological studies. This paper concerns results from the ground controls of ill-fated US Biosatellite A and compares them with results from other dose rates and types of radiation.

Males are packaged separately and used for mutational studies to be reported elsewhere. To compare the vulnerability of cell types in the ovariole sequence, nearly 1000 virgin females from a vigorous outcross are used, half for ground controls and half for the satellite launched. Samples of 20-25 wasps are packed in each of two screw-capped capsules inserted into housings in plastic modules which also incorporate thermistors and radiodosimeters. These packages are fixed in shielded positions as well as in places which receive one of four levels of gamma rays from the ^{85}Sr source. Each treatment thus consists of 40-50 virgins; a similar number receives a sensitizing preflight exposure to 2000 R of X-rays. After the flight period daily egg production is scored to detect resorption following gross chromosomal damage, embryonic deaths to reveal more subtle damage, and maternal life span as a measure of somatic fitness.

In most insects bundles of numerous ovarioles confound interpretation relating cell status during exposure to eggs deposited subsequently. Habrobracon's four synchronized ovarioles provide a uniquely suitable system for studying radiosensitivity of a sequence complete from specialized oöcytes through oöcyte-trophocyte units to primitive interphase cells. Following a series of doses, the family of oviposition curves reflects the vulnerability of differentiating units in a valley which deepens and broadens with increased dose. At high dose rates, lowest egg production occurs on day 7. The pattern, well established for X-rays and ^{32}P β-rays, has now been demonstrated for γ-rays from the Raleigh ^{60}Co Gammacell and the Woods Hole ^{137}Cs source, although 1.4 times the X-ray dose is required to produce the same amount of damage. When the gamma exposure was spread over three days the valley did not appear until the eighth day. Females tested simultaneously and treated identically except that their X-ray exposure was brief provided the usual 7th-day low. Previously valley deferment was observed only when studying ingested radioisotopes where a necessary prestarvation obscured the significance. Another unpredicted response to the protracted gamma dose was a control level productivity of eggs derived from exposed oögonia. Recovery mechanisms were evidently able to keep pace with radiation damage. On the other hand, embryonic lethality was related to dose during all periods studied and dose rate was not important except for the most mature oöcytes. As scored by von Borstel categories, stage I deaths decreased and stage III types increased with age of the mothers until the latter predominated. This change is age-related and not dose-dependent. Biochemically, predominance of stage III death can be induced, even in eggs from young mothers, by feeding either RNA or protein inhibitors, but not by interfering with DNA synthesis. We postulate physiological involvement of nurse cells and fat body or their interrelations. Mean life span ranged from 19.52 ± 1.34 days after 4320 R to 22.35 ± 0.91 for controls.

Biosatellite B, the second effort, was recovered successfully. After two days in orbit the reproductive performance of females differed strikingly from that repeated in ground controls. Most significant was egg deposit in a nearly contourless pattern reminiscent of a compensatory response to mitotic inhibition.

The release of "sterile" females is often a feature of experimental insect control. They may serve as an infertile siphon for sperm from fertile males or they may be a convenient way to avoid sexing a large sample. For an understanding of the sterilization of female insects it is important to distinguish between the agent's effects upon egg production and its effects upon the hatching of any eggs laid. These types of responses to radiation depend upon differences in the vulnerability of a variety of cell types.

The greatest variety of cell types occurs in the polytrophic ovariole which is the functional unit typical of the ovaries in holometabolous orders and a few others as well. In the polytrophic type of egg-forming tube, specialized nurse cells accompany each oocyte and both the differentiation and the functioning of the entire follicle-enclosed unit are important. An investigation merely concerned with scoring viable offspring provides no basis for analysis of the cytological action of the agent employed.

Even when egg production is recorded, the pattern of response may be obscured by an unsynchronized ovariole performance. This is likely to occur in species characterized by large bundles of egg tubes when they engage in continuous oviposition. A favorable system for studying radiosensitivity of the ovariole cell sequence is provided by a small number of synchronized ovarioles per ovary. The parasitoid wasp Bracon hebetor=Habrobracon juglandis offers such an advantage with only two synchronous ovarioles per ovary.

This year we had the opportunity to use braconids in experiments with Sr^{85} gamma ray sources which provided dose rates and exposure times differing from those usually used in insect radiobiology or ecological studies. The radiation sources were installed in U. S. biosatellites A and B and duplicate sources were employed for ground controls. In addition to 2 and 3 day gamma ray exposures a brief preflight exposure to X-rays was given to a group of wasps. This paper compares reproductive performance for biosatellite experiments with results for experiments using other dose rates and types of radiation. Criteria of damage scored include the pattern of egg production, egg hatchability, and the stage of development achieved before embryonic death. Since records are obtained from youth into senility, present results throw light upon the influence of aging on reproductive performance. Space flight introduces additional experimental conditions to which biologists have given little attention (mechanical vibration) and for which there has not been study opportunity (low gravity).

Of broad interest in bioastronautics is the general question of the performance during space flight of progenitive tissue with a stem cell component. The insect ovariole with its single series of units including all stages from fully differentiated products (the eggs) to interphase oogonia seems ideally suited for the studies. Eggs are dispensed in order and those deposited at any designated time can be traced back to their cytological condition during the exposure period. Quantitative modifications in numbers of eggs or hatchability may thus be correlated with the cellular state and critical periods of sensitivity identified.

MATERIALS AND METHODS

A vigorous wild type Bracon stock collected in Raleigh, North Carolina provided females used in the first biosatellite attempt

(December 14-17, 1966). Phenotypically wild type females heterozygous for three linked marker genes provided females for the second experiment (September 7-9, 1967). The genetic background came from a wild type stock collected in Lumberton, North Carolina.

Newly isolated virgin females were packed in screw-capped plastic capsules incorporating glass rod dosimeters. In turn the capsules were inserted into housings in plastic modules containing thermistors. The completed packages were fixed within the Experiment Capsule in positions aft of a radiation shield and a fore group arranged concentrically around the Sr^{85} source. Positioning was such that four different radiation doses were experienced by samples of wasps in the fore group of experiments. Each position held two samples of 20-25 wasps each so that 40-50 virgins experienced each treatment. Included in shielded position were females which had been X-rayed with 2,000 R. In analysis of data, homogeneity was ascertained before pooling the replicated data for presentation of results. Ground controls duplicated the shielded and exposed position for samples of virgins. Only the ground controls were available for study when Bios A failed to respond to retro-rocket firing command. Both ground control and flight animals were obtained from the second attempt, Bios B.

During the experiments females were maintained at 19-21° C. After recovery they were provided with a constant supply of host caterpillars and incubated at 30° C. During the first day, transfers were made every two hours for a study of genetic damage to metaphase and prophase oocytes by R. C. Von Borstel and R. H. Smith of Oak Ridge. Subsequently, the collection of eggs and provisioning with a fresh host caterpillar per female followed a daily schedule. Routinely eggs were immersed in mineral oil for determining their hatchability. This is an empirical method which not only minimizes variability but also expedites scoring inviable embryos by Von Borstel [1] categories. The stage of development achieved in each unhatched egg was determined. Life span was recorded for each of the ovipositing females in control and experimental groups.

RESULTS

EGG PRODUCTION

Bios A Ground Controls

After irradiation the more sensitive cells deteriorate and debris is resorbed. Subsequently, egg deposit is decreased on the day for which the resorbed cells would have produced eggs. Typically the ovariole response to an acute dose of ionizing radiation has been in the form of a trough or valley wherein lowest egg production was achieved by the seventh day of oviposition. In the Bios A experiment this response was demonstrated for the 2,000 R X-ray exposure given before packaging of a group of females (Fig.1, upper).

A consistent difference in oviposition pattern resulted from spreading doses of Sr^{85} gamma rays over three days of continuous exposure. As shown in Fig.1 (lower), the valleys characteristic of radiation damage developed their low points on the eighth day of oviposition. The entire family of curves shows this displacement or deferment in the pattern of response. In descending order 3-day gamma ray doses were 4,320 R, 2,410 R, 1,320 R, and 650 R obtained by position

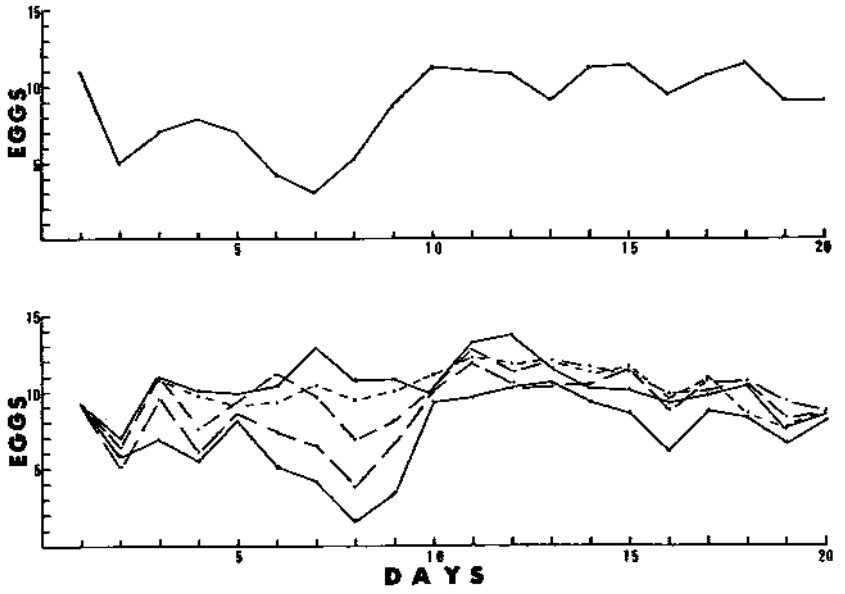

FIG. 1. Mean daily egg production plotted for samples of 40 braconid wasps. The upper graph gives the pattern of response to an acute dose of 2000 R of X-rays. The lower one plots data from unirradiated controls and from four doses of ^{85}Sr gamma rays delivered over a three-day period. The family of curves showing an eighth day dip was obtained after 650, 1340, 2410, and 4320 R, respectively.

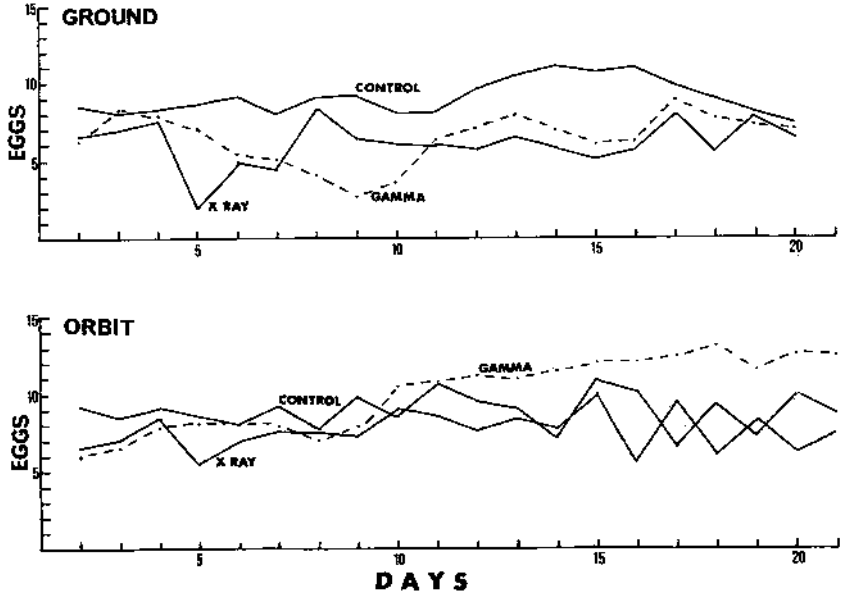

FIG. 2. Mean daily egg production for samples of female braconids subsequent to removal from the ground experiments vehicle (upper graph), and subsequent to recovery from Biosatellite B (lower graph). The latter wasps had experienced 30 orbits 170 miles in space. In each case curves have been drawn for unirradiated controls, for wasps receiving an acute preflight dose of 2000 R of X-rays, and for wasps receiving 2667 R in a gamma exposure spread over two days.

of the package in respect to the source. The top solid line gives the control record. Except for displacement of the low point, the curve shape appears to be essentially unchanged. During the last ten days of study egg production approached or exceeded control values.

Bios B Ground Controls and Flight Wasps

Oviposition records from females packaged but held on the ground at Cape Kennedy again demonstrated deferred low egg production for wasps receiving protracted doses of gamma rays. The valley did not reach its low point until the ninth day. Fig.2 (upper) contrasts the daily egg production pattern for females receiving the highest gamma dose of 2,667 R in a two-day exposure with data obtained from a brief exposure to 2,000 R of X-rays. Data from controls are also plotted.

Wasps recovered after a two-day space flight show a strikingly different pattern of oviposition when their gamma ray exposure was spread over most of the two days in 170 mile high orbit. No true valley developed. Instead, after a sloping plateau egg production rose in a fashion tending to compensate for relatively low oviposition during the first week. Fig.2 (lower) shows this pattern along with plots of data from females receiving 2,000 R of X-rays before the flight. Data for unirradiated control wasps shows more than the usual variability when plotted.

A detail of the curve shape in Bios A results which is not typical of Bios B results is a slight second day drop resulting from transporting the females from Cape Kennedy, Florida to Raleigh, North Carolina by plane at winter temperature.

EGG HATCHABILITY

Bios A Ground Controls

In contrast to egg production records, embryonic lethality was not modified significantly by a low dose rate for gamma rays. These results are presented as bar graphs on Figure 3. In order to summarize on the basis of cell types exposed, egg hatchability data are pooled in groups organized with respect to the days of deposit. Metaphase eggs and oocytes in late prophase of first maturation division give rise to ova deposited on the first day. Eggs laid on days 2-5 were prophase oocytes during the exposure period. Differentiating cells of the transitional period give rise to eggs laid on days 6-10. Eggs deposited during subsequent periods were derived from cells which were oogonia when treated but we recognize that days 16-20 correspond to a period of maternal senility. On that basis we distinguish it from the 11-15 day period.

The solid bars show how control hatchability decreases with the age of the mother. The diagonally shaded bars represent results from the X-rayed females. Through the important two weeks of the experiment from day 2 through day 15, hatchability after the acute 2,000 R did not differ significantly from the protracted dose results after 2,410 R of gamma rays. The 2,410 R results are represented by the open bar to the left of the shaded bar. Other open bars demonstrate the dose dependent pattern of egg hatching. In each grouping by days the dose decreases from left to right.

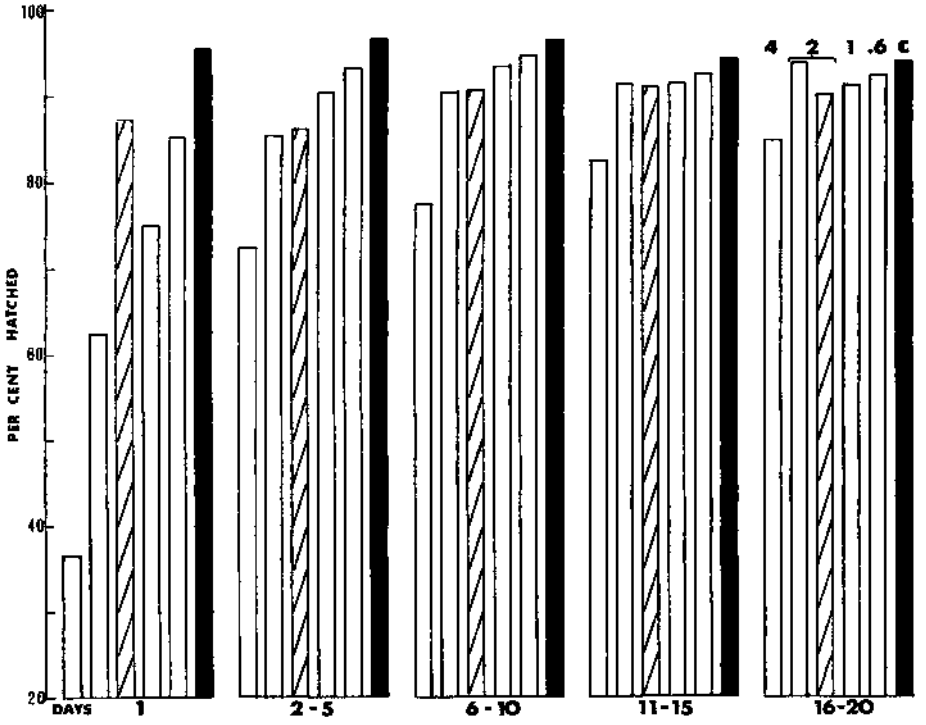

FIG. 3. Bar graphs summarizing hatchability for the eggs of Fig. 1. A key to dosages in R is given at the upper right: -4 = 4320, 2 = 2410 gamma and 2000 X-rays, 1 = 1340, .6 = 650, and C = control. The shaded bar identifies results after 2000 R of X-rays. As explained in the text, data are grouped into periods which reflect the cytological stage at the start of exposure.

Bios B

Analysis of hatchability of eggs laid by Bios B females is still in progress at the time of writing this preprint document. Nevertheless, the excellent hatchability evident in inspection of the raw data provides no indication of synergistic damage from space flight conditions in combination with radiation.

Stage of Embryonic Death

Immersion in clear oil and the thin (2μ) transparent chorion of the braconid egg make it possible by microscopic examination to determine the stage of development achieved by unemerged embryos. Von Borstel's laboratory [1] has classified stages of embryonic death into five categories. In our scoring of unhatched eggs, stages 1, 3 and 4 accounted for nearly all deaths in Bios A eggs (see Table I). Stage 1 deaths occur before blastoderm formation. State 3 shows that yolk has changed from white to yellow and is becoming localized. Stage 4 has developed to the point where tracheal elements and urate cells are visible.

Stage 1 deaths decreased and stage 3 types increased with age of the mothers until the latter predominated. Stage 1 showed the dose dependent trend except during the period of maternal senility (Days 14-20). On the other hand stage 3 proved to be primarily age related. Figure 4 provides an impressive demonstration of the increased predominance of stage 3 deaths, represented by the open circles. The triangles indicate the percentage of unhatched eggs which failed to progress beyond stage 1. Interestingly enough, the fifth type of lethal syndrome in which braconid embryos die late in development and which has been recognized as an enhancement of aging processes [1] did not contribute significantly to lethality in present results.

The statistical analysis of results from Bios B is not yet complete, but again age dependent stage 3 types predominate in lethality records after the first week.

LIFE SPAN

Table II presents a summary of life span for the Bios A females. Evidently conditions of the experiment including handling and packaging do not shorten life span appreciably.

With Bios B females 100% survival was obtained in both ground controls and flight groups. Furthermore with rare exception all these females survived more than 20 days of study. Final calculation of mean life span for these wasps must be delayed until all of them are dead.

DISCUSSION

The characteristic shape of the egg production curve for irradiated mothers is two hills separated by a valley which broadens and deepens with increased dose. The hills respectively represent the eggs derived from cells which were differentiated oocytes with nurse cells and from cells which were interphase oogonia at the time of irradiation. Both conditions provide a measure of resistance to the cell destructive action of ionizing radiation but mutational damage is another matter entirely.

Complete sterilization of a female with polytrophic ovarioles requires a radiation dose adequate to guarantee destruction of all oogonia and to induce at least one dominant lethal change in all oocytes. Although oogonial destruction after 5,000 R of X-rays [2] results in a cessation of egg production during middle and old age, the more mature oocytes can develop into eggs after any irradiation short of massive doses in the "knock down" range. Sterilization can be accomplished at moderate doses only because 10,000 R is adequate to induce dominant lethality in all oocytes [3]. These lethal mutations are expressed as embryonic death.

The valley reflects the vulnerability of differentiating units. Five successive mitotic divisions are required to produce the 32-cell oocyte-trophocyte unit from a single oogonium. In addition endomitosis must be accomplished in nurse cells to provide the high polyploidy of the functional state. Both mitosis and endomitosis are vulnerable to radiation damage. However, changes in shape and position of the valley obtained from differing dose rates imply a certain resilience in the cell physiology concerned with normalizing chromosome activity after disruptive ionizations from irradiation.

TABLE I. STAGES OF DEVELOPMENT ACHIEVED BY Bracon EMBRYOS IN THE UNHATCHED EGGS OF THE BIOS A EXPERIMENT. PERCENTAGES OF THE TOTAL DEAD.

Days	2-5			6-10			11-15			16-20		
Stages	1	3	4	1	3	4	1	3	4	1	3	4
Control	30.0	40.0	30.0	19.0	73.0	7.9	4.6	82.3	13.1	1.1	94.5	4.4
3 day gamma												
650 R	45.1	40.8	14.1	8.6	81.5	9.9	4.2	84.2	11.7	3.5	84.8	11.6
1340 R	44.0	54.8	1.2	11.1	84.6	4.3	7.2	84.4	8.3	1.6	90.5	7.9
2410 R	53.7	42.0	3.3	21.6	76.7	1.7	7.5	82.0	10.6	1.3	86.3	12.5
4320 R	56.1	38.6	5.3	23.8	72.1	4.1	13.6	81.9	4.5	1.8	92.1	6.1
Acute X-ray												
2000 R	39.1	51.1	9.8	1.2	91.7	7.1	4.5	80.5	15.0	1.7	91.3	7.0

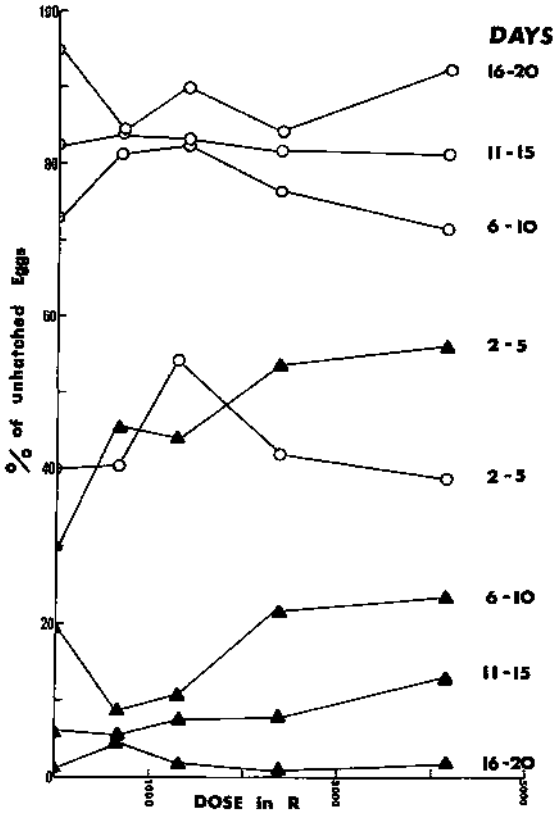

FIG. 4. The relative contribution to unhatched eggs by two stages of embryonic death in Bios A ground experiments. The percentage of deaths in stage 1 (triangles) and in stage 3 (circles) are plotted against dose and organized with respect to periods of deposit. Note that stage 1 deaths representing cleavage difficulties show a pronounced dose-dependent slope particularly for eggs from days 2-5. In contrast is the nearly horizontal distribution of stage 3 deaths which predominate later deposits.

Fractional dose experiments such as those used for studying chromosomal rejoining mechanisms in plants have provided some insight. By altering the length of the interfraction period [4] or the temperature during interfraction period [5] or by feeding inhibitors of protein synthesis [6] the derivation of eggs from oogonia was much altered. These treatments flattened the post-valley hill of the oviposition curve but did not shift the valley in time. Similarly although egg production changed quantitatively when Co^{60} doses required a day for their delivery [7] valley position was not shifted. Subsequently a variety of gamma sources including the Woods Hole Cs^{137} device has been used but no changes in valley position were obtained. The displaced valley reported herein resulted from spreading the gamma ray exposure over three days. This is nearly half of the important first week for insects which have an active life of little more than three weeks. Females tested simultaneously and treated identically except that their exposure was to X-rays and brief, provided the usual low at the end of the first week.

TABLE II. THE MEAN LIFE SPAN OF FEMALE BRACONIDS FROM BIOSATELLITE EXPERIMENTS

Treatment	December 1966	September 1967	
	Ground	Ground	Flight
Control	22.4 ± 0.9	23.3 ± 0.9	25.1 ± 0.7 32.1 ± 2.5[a]
Pre-X-rayed 2000 R	20.2 ± 0.9	21.9 ± 1.9	24.2 ± 1.5
3-day γ-rays 4320 R	19.5 ± 1.3		
2410 R	22.8 ± 0.9		
1340 R	21.8 ± 1.1		
650 R	20.8 ± 1.5		
2-day γ-rays 2350 R		24.9 ± 1.5	34.1 ± 4.4[a]
1200 R		23.8 ± 1.9	24.3 ± 1.4
700 R		17.2 ± 2.7	24.5 ± 0.5
		21.4 ± 2.3	
346 R		21.6 ± 1.8	23.3 ± 1.4

(a) Received special care during the last week of life.

Until now we had observed deferment of the valley only when studying females fed radioisotopes [8]. Here too the radiation dose accumulated over a period of days, but the significance of the observation was obscured because of the necessary prestarvation in preparation for ingestion experiments.

That there are efficient recovery mechanisms was revealed by control level production of eggs derived from oogonia of Sr^{85} exposed females. Presumably recovery mechanisms can keep pace even during irradiation and, if the dose rate is low enough, presumptive lesions can be corrected before the irrevocable biochemical events develop.

Cells differing in stage may be expected to differ in the efficiency of their recovery mechanisms. Also unless the system is completely at rest, certain cells may progress to a stage of lower vulnerability while others have entered a stage of high vulnerability before a critical dose has accumulated. The S stage of braconid interphase oogonia has never been localized. Perhaps its occurrence is related temporally with the delayed valley.

In these considerations it is important to appreciate that cells do not necessarily die in the stage irradiated. They die when faced with an insurmountable cytological crisis such as the mitotic period and the endomitotic period necessary to differentiate the polytrophic contents of the ovariole. We are not concerned about the follicle because its cells come from a different germ layer and have proved relatively radioresistant.

In contrast to the radiation response a different type of pattern for the oviposition curve was obtained from females irradiated while in space flight. The curve most nearly resembles compensating ovipositional records obtained from feeding mitotic inhibitors [9]. This type of response did not appear either with centrifuged or vibrated females [10]. Therefore, we postulate an effect of low gravity on dividing and differentiating cellular units of the ovariole. A well established way to moderate radiation damage to chromosomes is the use of a mitotic inhibitor such as colchicine [11]. Good egg production and hatchability can be explained by low gravity contributing a similar influence.

Most of this discussion has centered on egg production. This is important not merely as the limiting factor to insect fertility but because it provides the same type of curves seen in vertebrate peripheral hematology [12] and in sperm studies of domestic animals [13]. Now we shall briefly consider matters which become evident after eggs are deposited.

Considerable information is already available on egg hatchability especially for eggs laid during the first week [1]. An additional contribution to our growing understanding of embryonic survival is demonstration of the predominance of stage three deaths when mothers have been ovipositing for a week or more. The change is age-related and not dose-dependent. Depletion of the abdominal fat body and accumulation of urate deposits characterize actively ovipositing braconids. Under such circumstances, although the abdomen's important somatic tissues can sustain life for a normal span, their efficiency in supplying nurse cells with the materials needed for oocytes may decline after a week or more of peak operation. This could mean that compensatory oviposition in flight animals would jeopardize eggs to age defects

by causing more eggs to be deposited late in the mother's life. Even before oviposition began stress on tissue reserves was experienced by both ground controls and flight wasps since they were packaged without a food supply.

Stage three deaths indicate that the embryo was able to accomplish cleavage and form the blastoderm but lacked either the information or the materials to proceed in development. Nuclear imbalance has characterized genetically contrived stage three lethals but we are inclined toward cytosomal defects in explaining the prominance of the syndrome in Bios experiments. Radiation injury need not enter the explanation. A. R. Whiting [14] has demonstrated that cytoplasmic injury does not kill eggs at the doses employed here. Results with chemical agents have strengthened our position. A predominance of stage three deaths can be induced even in young mothers by administering inhibitors of RNA as well as by agents which cause DNA cross linkage [15]. Perhaps cell division and differentiation is proceeding at a faster rate in the ovarioles than the somatic tissues of an aging insect can manufacture and transfer materials to the ovary.

ACKNOWLEDGMENTS

This work has been supported by N.A.S.A. Research Grant NsG 678. It could not have been performed without the cooperation of Dr. R. C. Von Borstel and Dr. R. H. Smith of Oak Ridge National Laboratory nor without assistance from Mrs. Ruth Ann Carpenter, A. C. Hoffman, L. R. Valcovic and many others from the Raleigh campus.

SUMMARY

Three criteria of damage can be employed in radiation experiments with female braconids. Egg production records provide a measure of the gross chromosomal and nuclear damage to a variety of cell types. Egg hatchability reveals more subtle damage, both genic and age related. Life span records reflect the ability of somatic tissues to function for general body maintenance.

The shape of the egg production curve is discussed from the standpoint of appreciating the cytology of the insect ovariole. Hatchability data are analyzed with respect to the stage of embryonic development achieved in unhatched eggs. Large scale experiments performed in conjunction with the first and second U. S. biosatellite experiments provided striking evidence of

(1) a deferred low period of egg production from protracted gamma ray doses delivered in ground experiments,

(2) no pronounced low for females recovered from space flight,

(3) both dose-dependent and age-influenced components of embryonic lethality,

REFERENCES

[1] VON BORSTEL, R. C., REKEMEYER, M. L., Genetics 44 (1959) 1053;
VON BORSTEL, R. C., St. AMAND, W., Repair from Genetic Radiation (SOBELS, F. H., Ed.) Pergamon, Oxford (1963) 8.

[2] GROSCH, D.S., SULLIVAN, R.L., Radiat.Res. 1 (1954) 294.
[3] HEIDENTHAL, G., Genetics 30 (1945) 197.
[4] GROSCH, D.S., p. 83 Annual Review of Entomology (STEINHAUS, E.A., SMITH, R.F., Eds) Annual Reviews Inc., Palo Alto, California (1962).
[5] GROSCH, D.S., Biological Effects of Radiations, Blaisdell/Ginn, Waltham, Mass. (1965) 174.
[6] GROSCH, D.S., Radiat.Res. 14 (1961) 470.
[7] GROSCH, D.S., Radiat.Res. 9 (1958) 123.
[8] GROSCH, D.S. et al., Radiat.Res. 5 (1956) 281.
[9] GROSCH, D.S., Ann. ent. Soc. Am. 52 (1959) 294.
[10] GROSCH, D.S., 3rd. Int. Congr. Radiat. Res. (1966) Abstract 390.
[11] LEA, D.E., p. 220 Actions of Radiations on Living Cells, Cambridge Univ. Press (1947).
[12] JACOBSON, L.O., Hematologic Effects of Ionizing Radiation, Ch. 16: Radiation Biology (HOLLAENDER, A., Ed.), McGraw-Hill, New York.
[13] GILLETTE, E.L. et al., Radiat.Res. 22 (1964) 264.
[14] WHITING, A.R., Radiat.Res. 2 (1955) 71.
[15] SMITH, R.H., Ph.D. Thesis, N.C. State University (1965) Raleigh, N.C., USA.

DISCUSSION

K. K. NAIR: Could you give some details on the levels of cosmic radiation inside the space capsule in your experiments and explain whether you observed any increase in the frequency of recessive mutations?

R. C. VON BORSTEL: If I may answer the first part of this question, the orbit of Biosatellite II was below the Van Allen Belt, and hence no radiation from that source contributed significantly to the effect. In fact, radiation from heavy cosmic elements, as measured dosimetrically on the spacecraft, was negligible. The recessive lethal mutation data are still under analysis.

D. S. GROSCH: With the postponement of Biosatellite II until September, the 20-day oviposition experiments were barely completed before the deadline set for submission of papers to this Symposium. Analysis of recessive lethals necessitates study of generations subsequent to the F_1 and so this work is as yet incomplete.

GENETIC AND CYTOGENETIC BASIS OF RADIATION-INDUCED STERILITY IN THE ADULT MALE CABBAGE LOOPER Trichoplusia ni

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Abstract

GENETIC AND CYTOGENETIC BASIS OF RADIATION-INDUCED STERILITY IN THE ADULT MALE CABBAGE LOOPER Trichloplusia ni. The relationship of egg hatch as a function of radiation dose is of a two-hit nature when irradiated adult males are mated to non-irradiated virgin females. Sterility as a function of dose is usually linear in insects. However, other species of moths respond similarly to irradiation, so it is indicative that the mechanism involved in producing sterility in Lepidoptera is basically different from that in other insects. The significance of the two-hit kinetics is discussed in relation to the chromosome structure and possible mechanisms for the induction of sterility in Lepidoptera. Many workers using Lepidoptera have reported that females mated to irradiated males oviposit substantially fewer eggs than normally. This response has been correlated to a lack of sperm transfer by irradiated males, even though they pass a spermatophore. The phenomenon is dose-dependent. Although Lepidoptera are far more radioresistant than other insect species when measured by the induction of sterility in the male, there appears to be very little difference when longevity is used as the criterion. The radiation dose required to reduce the lifespan of a newly emerged cabbage looper male by 50% was found to be approximately the same as that for the house fly. High doses of radiation have no immediate effect on the mating behaviour of the irradiated male. With a recessive eye-colour mutant as a sperm marker, it was determined in tests utilizing double matings that the second mating is the effective mating. Sperm mixing is not prevalent; rather it appears to be a "sperm flushing" phenomenon in that sperm from the first mating are displaced by sperm from the second mating. Radiation studies with the cabbage looper have demonstrated that the progeny of a cross where the male parent receives a sub-sterilizing dose of gamma radiation are often semi-sterile when mated to non-irradiated individuals. The amount of inherited sterility is directly dependent on the amount of radiation given the original parent. A dose of 10 krad to a P_1 male, for example, only induces 15 to 20% sterility. However, of the surviving progeny as many as 50% will be semi-sterile with 20% being completely sterile when mated to non-irradiated individuals. The cytogenetic implications of this are discussed; namely, the effect of diffuse centromeres, and the induction of reciprocal translocations. Data are presented on induced translocation frequencies by various doses of radiation and the F_2-bred behaviour of these individuals. This approach possibly affords a more effective tool in insect control.

1. INTRODUCTION

The success of the sterile-male technique in eradicating or controlling the screw-worm fly, Cochliomyia hominivorax (Coquerel), [1, 2] has led entomologists to increase their efforts to apply this technique to many other insects. Such releases of sterile males into natural populations have been effective with many species of Diptera [3-7], but to date have not had great success with species of Lepidoptera. One difficulty is that Lepidoptera are highly resistant to irradiation when the criterion is induced sterility, e.g. 30-40 krad are required to sterilize the codling

moth Carpocapsa pomonella L. [8], the European corn borer Ostrinia nubilalis (Hübner) [9], the cabbage looper Trichoplusia ni (Hübner) [10], the tobacco budworm Heliothis virescens F. [11], and the sugar-cane borer Diatraea saccharalis F. [12], and 100 krad are needed for the Indian-meal moth Plodia interpunctella (Hübner), and the Angoumois grain moth Sitogroga cerealella (Olivier) [13]. About a tenth as much is required to induce male sterility in Dipteran species, and most require only 4-5 krad administered to the adult, e.g. the screw-worm [14], the Mexican fruit fly Anastrepha ludens (Loew) [15], and the house fly Musca domestica L.

Insects that require 30 krad or more to induce sterility are more likely to incur severe physiological and somatic damage than are more radiosensitive species. In particular, the higher dose makes irradiated males much less competitive than the unirradiated males in a wild population. Successful application of the sterile male technique depends on the production of sterile males that are competitive enough to decrease the reproductive potential of the population into which they are released. Thus, realization of this objective depends on studying and understanding the effects of irradiation on the reproductive biology of the insects. With Lepidopteran species it is a distinctly more difficult problem than with Dipteran species, where sterile males are usually competitive. Only after the various factors influencing competitiveness in Lepidoptera are known can the factors responsible for non-competitiveness be overcome. The studies reported here are therefore an attempt by the authors to evaluate the factors influencing the sterility and competitiveness of the cabbage looper and to suggest solutions to problems encountered with this and other Lepidopteran species.

2. MATERIALS AND METHODS

The cabbage looper strain used in these experiments was originally obtained from the laboratory of the Fruit and Vegetable Insects Branch of the US Department of Agriculture at Riverside, California; it has since been maintained at Fargo through more than 30 generations. All insects were reared individually in 1-oz plastic jelly cups on a semi-synthetic medium [16]. The adults were fed a 10% solution of sucrose and maintained at 27.5 ± 2°C with a 12-h photoperiod and a relative humidity of about 75%. Males irradiated as 2- to 4-day-old adults were used in single-pair crosses with unirradiated virgin females. The holding cages were mesh-screen cylinders (7.5 × 17.5 cm). After one night, the males were removed and the females were allowed to oviposit on wax paper that was placed around the outside of the cages each day. The next day, any damaged eggs were removed, and the others were gently brushed from the paper and placed in rows on damp, sterile black muslin or on black filter paper in sterile petri dishes. The petri dishes were than placed in sealed plastic bags, with a wet paper towel around every two dishes to prevent desiccation, and incubated for three days at 28.5°C. After incubation, the petri dishes were examined under a dissecting microscope, and the percentage of hatched eggs was determined. The resulting larvae were transferred to larval diet cups if the progeny were to be recovered.

Tests of competitiveness were made with one irradiated male, one unirradiated male and one virgin female. Other conditions were identical to those described except that the males were not removed from the cages until the end of the 5- to 7-day test. In these experiments, the 2- to 4-day-old adult unanaesthetized males were irradiated with gamma rays from a cobalt-60 source at a dose rate of 7440 rad/min. Since only adult males were irradiated, mature sperm was the only stage tested.

All females were dissected after the study and the number of spermatophores in the bursa copulatrix were recorded, as was the presence or absence of sperm in the spermathecae (determined by squashing the spermathecae into physiological saline solution and by microscope examination). All cytological observations were made by squashing the testes of late-instar larvae and staining with aceto-orcein.

3. RADIATION-INDUCED MALE STERILITY

Thirty krad of gamma radiation causes nearly complete sterility in the adult male cabbage looper (Table I). The dose-response curve for induced male sterility is non-linear (Fig. 1) instead of linear as in most insect Dipteran species (Fig. 2). No significant sterility occurs in the cabbage looper until a dose of over 10 krad is administered, but sterility thus increases rapidly until little hatching occurs. Thus dose-response curves for male sterility in the cabbage looper and other Lepidoptera are distinctly different than for Diptera. The cabbage looper is apparently more resistant to radiation-induced sterility than the house fly, and the origin and nature of the sterility are also different.

The dose-response curve of the cabbage looper, and probably that of all Lepidoptera, is basic to the use of these species in sterile male releases. Since large doses of irradiation are required to induce sterility, the lowest possible dose must be selected. For example, if a house fly is overdosed by as much as 20%, only an additional thousand rad or less are administered. If the cabbage looper is overdosed by the same percentage, an additional 8000 rad of radiation are absorbed and so large a dose may cause serious physiological damage including the loss of competitiveness in the sterile male. In addition, as Fig. 1 shows, administration of 40 instead of 30 krad does not increase the amount of induced sterility appreciably but represents a 25% overdose.

4. EFFECT OF RADIATION ON LONGEVITY

Although the cabbage looper is far more resistant to induced sterility than the house fly, the reduction of longevity caused by irradiation of both species with 50 krad was about the same (Fig. 3). Since 50 krad is only 20% higher than the sterilizing dose for the cabbage looper and since the longevity of the house fly is little affected at the 4-krad sterilizing dose, the cabbage looper is comparatively radioresistant in terms of sterility but not in terms of longevity.

Reduced longevity has variable effects on the efficiency of sterile males (a detailed discussion is beyond the scope of this paper), but in polygamous species such as Lepidoptera it has an obvious effect. If

TABLE I. THE RELATION OF GAMMA IRRADIATION DOSE TO INDUCED STERILITY IN 3-DAY-OLD ADULT CABBAGE LOOPERS AS DETERMINED BY INDIVIDUAL PAIR MATINGS (TREATED MALES X UNTREATED FEMALES)

Dose X 10^3 rad	Number of eggs	Number of eggs hatched	Per cent hatch ± S.D.
0	10261	9287	90.5 ± 5.9
1	2896	2662	91.9 (a)
2	4577	4163	90.0 ± 3.2
3	2550	2279	89.4 (a)
4	5285	4620	87.0 ± 5.0
5	6277	5227	83.4 ± 1.5
6	3403	2919	85.7 ± 1.5
7	3122	2583	82.7 ± 2.9
8	3589	3055	85.1 ± 0.92
9	1712	1250	73.0 ± 5.3
10	8442	5006	59.3 ± 9.8
12	2522	1557	61.7 ± 0.85
15	2921	1590	54.5 ± 0.80
18	1324	291	22.0 (a)
20	175	26	14.9 ± 3.5
25	2136	132	6.2 ± 4.6
30	3713	131	3.5 ± 2.1
35	2463	107	4.3 ± 0.70
40	1137	0	0 (a)
50	544	0	0 (a)

(a) Only one replication.

sterile males only lived long enough to mate once, then either a large number of males would have to be released or frequent releases would have to be made.

5. EFFECT OF RADIATION ON THE ABILITY OF THE MALES TO MATE

The effect of reduced longevity on the mating ability of sterile males was studied to determine its importance. The 3-day-old male cabbage

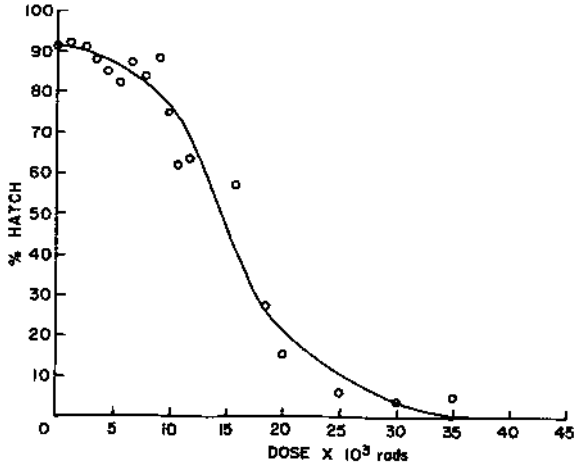

FIG. 1. The relationship of radiation gamma dose to induced male sterility (2- to 4-day-old irradiated males crossed individually with non-irradiated virgin females).

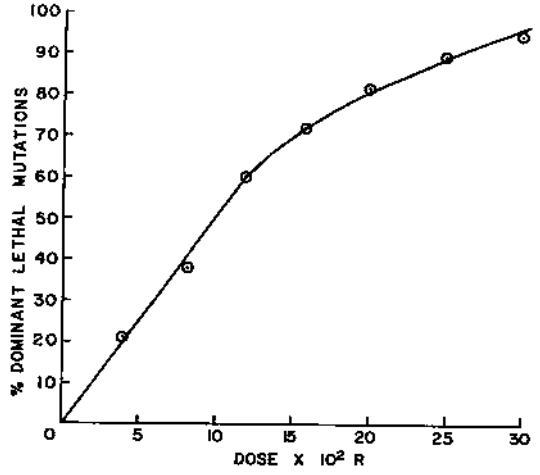

FIG. 2. The relationship of X-ray dose to the frequency of induced dominant lethal mutations in 3-day-old adult male house flies.

loopers were irradiated (0-50 krad) and placed individually with virgin females for one night; mating was determined by dissecting the females the following day to determine the presence of a spermatophore. The ability of the male to mate was not initially impaired, even after 50 krad of radiation (Table II). However, the data do not measure the mating ability of the irradiated male over a longer period of time, and tobacco budworm males that received 40 krad were 20% less effective in mating than the controls over the lifetime of the males [11].

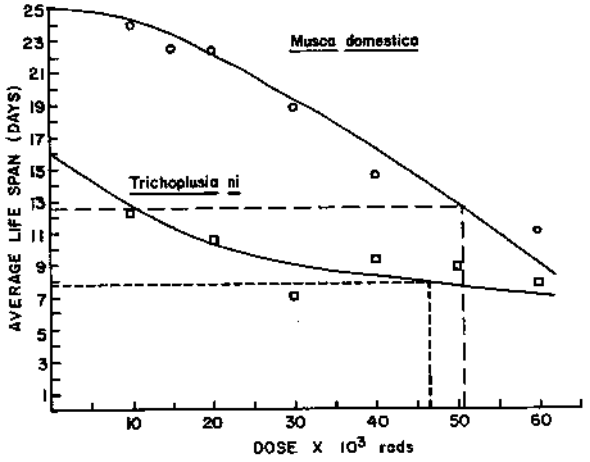

FIG. 3. The effect of life shortening by gamma radiation for 1-day-old adult cabbage loopers and house fly males.

6. OVIPOSITION ELICITED BY IRRADIATED MALES

A common observation by investigators of Lepidoptera has been the failure of the irradiated male to elicit normal oviposition in matings with unirradiated females. For example, unirradiated cabbage looper females mated to males that had received a sterilizing dose of 30 krad oviposited 50% fewer eggs than the controls (Table III). This reduced oviposition affects the evaluation of the competitiveness of sterile males. If mating competitiveness is equal, that is, if one-half the females mate with sterile males, the egg hatch will be 66%, not 50% of normal (based on 100% for the normal egg hatch of an unirradiated male mated with an unirradiated female and 0% for the hatch resulting from the cross of a sterile male and an unirradiated female). Therefore, the sterile male may be mating competitively though he is unable to reduce egg hatch effectively. Unless this fact is established, lack of competitiveness might be attributed to a lack of mating ability which would be unfounded.

7. SPERM UTILIZATION

Since the cabbage looper is a polygamous species, we examined the sperm-utilization pattern of females mated with both sterile and unirradiated males. Such information is needed to evaluate the effect of sterile male release into a population on the reduction of that population's reproductive potential. The effect the sterile male has on the natural population depends on whether sperm mix in the spermthecae after multiple matings or are used preferentially. Thus egg hatch depends on whether sperm are used randomly in fertilizing eggs or whether sperm from only one mating are used.

Sperm utilization in the cabbage looper was therefore investigated by using a recessive yellow-eyed mutant as a sperm marker. The

TABLE II. THE EFFECT OF GAMMA RADIATION ON THE MATING RESPONSE OF 3-DAY-OLD ADULT MALE CABBAGE LOOPERS AS DETERMINED BY INDIVIDUAL PAIR MATINGS (TREATED MALES X UNTREATED FEMALES) FOR ONE PERIOD OF 24 h

Dose X 10^3 rad	Per cent males mated
0	50.00
1	66.67
2	62.50
3	63.16
4	63.64
5	55.88
6	69.23
7	54.55
8	46.67
9	61.54
10	69.23
12	64.28
15	56.25
18	66.67
20	40.00
25	66.66
30	65.52
35	46.43
40	77.27
50	64.28

homozygous yellow-eyed females were alternately mated for one night each to normal-eyed males and yellow-eyed males, and the progeny were recovered. Only the progeny from females having two spermatophores and sperm in the spermathecae were collected. Since cabbage loopers rarely mate twice the same night, the use of spermatophore counts to determine the number of matings appears valid. Since the mutant is recessive, any sperm bearing the mutant phenotype produced a yellow-eyed individual. Sperm possessing the wild-type allele (normal eye) produced progeny having normal eye colour.

When a normal male was the first mate and the second was a yellow-eyed male, about 85% of the resulting progeny had yellow eyes. If the first mating was with a yellow-eyed male and the second with a normal-

TABLE III. RADIOSENSITIVITY OF 2- TO 3-DAY-OLD MALE CABBAGE LOOPERS DEFINED BY DEPRESSED FECUNDITY AS DETERMINED BY INDIVIDUAL PAIR MATINGS (TREATED MALES X UNTREATED FEMALES)

Dose X 10^5 rad	Number of females	Number of eggs	Number of eggs/female	Avg. eggs/oviposition
Control	22	10 261	466.4	148.7
1	5	2896	579.2	144.8
2	10	4577	457.7	143.0
3	5	2550	510.0	127.5
4	14	5285	377.5	101.6
5	18	6277	348.7	125.5
6	9	3404	378.0	85.08
7	11	3122	283.8	120.1
8	7	3589	512.7	143.6
9	8	1712	214.0	85.6
10	27	8442	312.7	99.3
12	10	2522	252.2	93.4
15	16	2921	182.6	104.3
18	5	1324	264.8	88.3
20	4	175	43.8	21.9
25	8	2136	267.0	46.4
30	19	3713	195.4	74.3
35	13	2463	189.5	61.6
40	22	1137	51.7	24.7
50	14	544	38.9	20.1
Virgin	159	2230	14.0	10.0

eyed male, 98% of the resulting progeny had normal eye colour (Fig. 4); that is, the last mating was the effective mating.

8. SPERM TRANSFER BY IRRADIATED MALES

If the conclusions concerning polygamy are valid, the irradiated male in a test of mating competitiveness must contribute a physiologically normal (also adequate in numbers) complement of sperm at each mating.

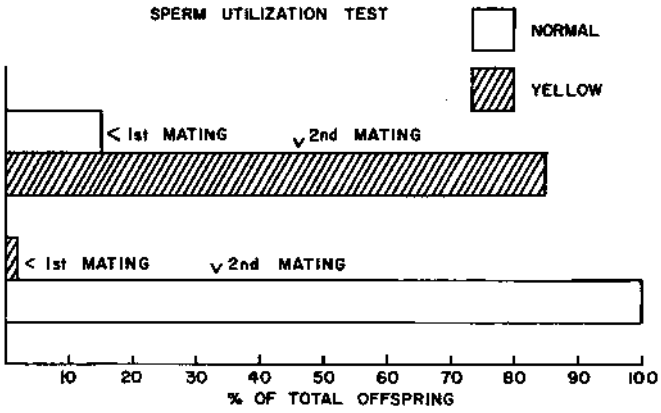

FIG. 4. The utilization of sperm from two different matings by female cabbage loopers (all were mated to ye/ye and +/+ males. The resulting progeny are indicated by phenotype indicating the source of the sperm used).

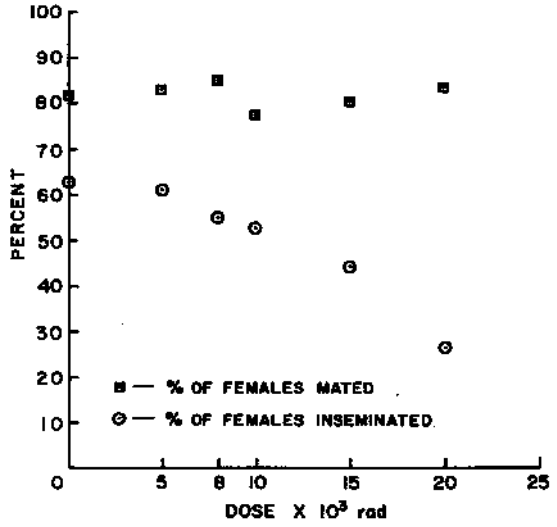

FIG. 5. The relationship of mating to insemination of females by irradiated male cabbage loopers.

In Atteva punctella (Cramer), the primary stimulus for oviposition is the successful transfer of an adequate supply of sperm [17]. This was true for the cabbage looper; however, irradiated males may transfer only spermatophores at mating, and they often failed to supply an adequate amount of sperm or any sperm since this transfer is dose-dependent (Fig. 5). Male cabbage loopers irradiated with 0-25 krad mated as well as the unirradiated controls, but as the dose increased, the percentage of males that transferred sperm capable of reaching the spermathecae of the female decreased rapidly. At 20 krad, less than 30% of the males successfully transferred sperm to the female compared

with 63% of the unirradiated males. This failure to inseminate adequately explains why irradiated males fail to elicit normal oviposition from unirradiated females. Thus the lowest irradiation that will sterilize the male must be used if a competitive sterile male is to be produced.

Studies by other workers with trap collections have shown that cabbage looper females in wild populations mate an average 2.5 times [18], as determined by spermatophore counts. Thus, if the sterile male contributes a normal complement of sperm at each mating, polygamy will not be a problem and should not detract from the effectiveness or efficiency of a sterile male release.

9. STERILITY IN THE F_1 GENERATION

The chromosomes of Lepidoptera have diffuse centromeres [19]. Such chromosomes would impart a high degree of radioresistance to the induction of sterility [10], because the loss of chromosomal material at cell division due to chromosomal fragments would not be expected and therefore should not lead to dominant lethality. Consequently, the typical chromosomal anomalies that lead to dominant lethal mutations in species that have monocentric chromosomes probably do not cause dominant lethal mutations in Lepidoptera.

However, the diffuse centromere also allows a source of sterility not available in many other orders of insects. The basis for this unique transmissible sterility is given below.

In addition to the loss or retention of chromosome fragments, we must consider the consequences of rejoining of the broken chromosomes and its effect on insect sterility. Broken chromosomes can reunite in many ways [20]. One type of chromosome rearrangement involving non-homologous chromosomes yields one or two acentric fragments (without centromeres) and chromosomes with two centromeres (dicentric). At anaphase of the next somatic division, a dicentric chromosome produces a chromosome bridge which, when broken, results in daughter cells that are deficient for some portion of the genetic material and possess some duplicated chromosomal segments. The result is cell death – a cytogenetic basis for the lethal action of dominant lethal mutations. In Lepidoptera, dicentrics of this origin possibly do not exist because every such exchange is essentially a reciprocal translocation. Most reciprocal translocations do not involve any appreciable loss of chromosomal material and are therefore transmitted to the offspring. Thus all of these types of rearrangements are transmitted to the progeny which can develop normally. However, at meiosis in the progeny, the inherited reciprocal translocation could result in the formation of germ cells that contain duplicated and deficient amounts of genetic material [21]. When these sperm are used in fertilization, they are lethal to the embryo for the same reasons that sperm containing a dominant lethal mutation impart death to the developing embryo in species without diffuse centromeres. The percentage of sterility in the F_1 generation caused by such a rearrangement is then dependent on the number of chromosomes involved in the exchanges. If parts of two different chromosomes are exchanged, 50% of the sperm or ova produced by an adult will be inviable.

A translocation involving parts of three different chromosomes theoretically will cause 75% sterility.

Thus, if several reciprocal translocations were induced in each sperm of the irradiated male parent, his progeny would be partially to fully sterile, and indeed, this occurs in the cabbage looper [10]. Large numbers of translocations were observed in the developing meiotic germ cells of F_1 males whose male parents received a sub-sterilizing dose of gamma irradiation. (The chromosomal configuration formed by such a reciprocal translocation can be easily detected cytologically in the testes of F_1 larvae.) The large numbers of heterozygous reciprocal translocations obtained with sub-sterilizing doses then explain the sterility observed in the F_1 generation. Since no sizeable number of dominant lethal mutations are induced by even relatively large doses of radiation, dominant lethal mutation in Lepidoptera cannot limit the recovery of the induced reciprocal translocations as they do in Diptera or in species having chromosomes with localized centromeres. In Dipteran species, for example, the induction of dominant lethal mutations in 100% of the sperm at relatively low doses (below 10 krad) limits the number of reciprocal translocations that can be recovered.

The diffuse (non-localized) centromeres of the cabbage looper offer the possibility of using the induced reciprocal translocations as a source of F_1 sterility and as an additional and somewhat unique tool to improve the efficiency of a sterile male release programme. A lower dose of radiation for adult males that are to be used in field releases would probably allow these males to be more competitive. The cross between a male that had received 20 krad and a normal unirradiated female would produce an egg hatch of 15-20% (Table IV). However, the progeny that survived to adulthood would be sterile. Furthermore, those progeny that did survive would be a component in the wild population of the next generation and would produce a population with inbuilt sterility. Success of such an application depends on many factors, not all of which are interrelated. For example, it has yet to be proved that the sterile F_1 male is competitive with unirradiated males. Also, methods of increasing

TABLE IV. INHERITED STERILITY OF PROGENY OF A MALE CABBAGE LOOPER THAT RECEIVED A SUB-STERILIZING DOSE OF RADIATION CROSSED WITH AN UNIRRADIATED VIRGIN FEMALE (F_1 EGGS OBTAINED BY CROSSING F_1 INDIVIDUALS TO UNIRRADIATED VIRGINS OF THE OPPOSITE SEX)

Dose received by P_1 male (krad)	Number of P_1 eggs	% hatch of P_1 eggs	Number of F_1 eggs	% hatch of F_1 eggs
Control	2783	85.6	2964	83.4
10	2338	46.0	8226	30.7
15	2068	39.5	5629	11.5
20	2345	15.7	1317	3.8

the induction of translocations with agents other than gamma irradiation are currently being examined. With increased information about the production of F_1 sterility, a practical and efficient method of using this procedure can be developed that will benefit any sterile male release programme for the control of Lepidoptera and for other insects having chromosomes possessing non-localized centromeres.

ACKNOWLEDGEMENT

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REFERENCES

[1] KNIPLING, E.F., J. econ. Ent. 48 (1955) 459-62.
[2] KNIPLING, E.F., Science 130 (1959) 902.
[3] STEINER, L.F., MITCHELL, W.C., BAUMHOVER, A.H., Int. J. appl. Radiat. Isotopes 13 (1962) 427-34.
[4] STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D., J. econ. Ent. 58 (1965) 519.
[5] CHRISTENSON, L.D., Insect Population Control by the Sterile-Male Technique, Technical Reports Series No.21, IAEA, Vienna (1963) 31.
[6] STEINER, L.F., Advances in Insect Population Control by the Sterile-Male Technique, Technical Reports Series No. 44, IAEA, Vienna (1965) 28.
[7] KATIYAR, K.P., Ibid., 20.
[8] PROVERBS, M.D., NEWTON, J.R., Can. Ent. 94 (1963) 1162-70.
[9] WALKER, J.R., BRINDLEY, T.A., J. econ. Ent. 56 (1963) 522-25.
[10] NORTH, D.T., HOLT, G.G., J. econ. Ent., in the press.
[11] FLINT, H.M., KRESSIN, E.L., J. econ. Ent., in the press.
[12] WALKER, D.W., Puerto Rico Nuclear Center Rep. 1 (1965).
[13] COGBURN, R.R., TILTON, E.W., BURKHOLDER, W.E., J. econ. Ent. 59 (1966) 682-85.
[14] BUSHLAND, R.C., HOPKINS, D.E., J. econ. Ent. 44 (1951) 725-31.
[15] RHODE, R.H., LOPEZ, D., EQUISA, F., TELICH, G., J. econ. Ent. 54 (1961) 202-03.
[16] IGNOFFO, C.M., Ann. ent. Soc. Am. 56 (1963) 178-82.
[17] TAYLOR, O.R., Jr., Ann. ent. Soc. Am. 60 (1967) 583-90.
[18] GENTRY, R., personal communication (Oct.1967).
[19] VIRKKI, N., J. Agric. Univ. Puerto Rico XLVII (1963) 102-37.
[20] LEA, D.E., Actions of Radiation on Living Cells, Cambridge Univ. Press, London (1946).
[21] PAINTER, T.S., MULLER, H.J., J. Hered. 20 (1929) 287-98.

DISCUSSION

C. F. CURTIS: I did not fully understand Table IV of your paper, showing the fertility of the P_1 and F_1 generations after irradiation. If, as I understand it, the result for the F_1, referred to eggs produced by the sons and daughters of the irradiated insects, I do not see why these eggs should not have exhibited the minimum level of fertility, since the effects of translocation would have shown themselves at this stage.

D. T. NORTH: One must bear in mind that in the case of sterility in the P_1 generation one is speaking of largely physiological damage together

with a certain amount of genetic damage, and in the F_1 generation one is speaking of mainly genetic damage. In a sense the dominant lethals, as they occur in species such as Diptera, are not fully recovered until the second generation in Lepidoptera.

A. B. BORKOVEC: I was interested in the statement that a radiation-sterilized male can reduce the fecundity of the female of the species. There is an analogy here with the observed effects of mating chemo-sterilized males of the azuki bean weevil (Collosobruchus chinensis) to untreated females. At first we thought that some of the sterilant was transferred from the male to the female during copulation and that the diminished fecundity was a direct effect of the chemical, but for various reasons this explanation is inadequate and I would like to know whether you have any data indicating that the drop in fecundity occurred only when there was no adequate sperm transfer or whether it occurred also when transfer was adequate.

D. T. NORTH: To the best of our knowledge, when a full complement of sperm is transferred and reaches the spermathecae of the female, she oviposits a normal number of eggs. It is only when she receives an inadequate amount of sperm or seminal fluid that her oviposition response is lowered.

V. LABEYRIE: Your hypothesis on the possible effects of elimination of certain secretions normally present in the seminal fluid corresponds to observations made on females mated with males which had already performed a certain number of copulations, or where copulation was artificially interrupted. All these experiments showed successive transfer of various substances during the period of formation of the spermatophore within the bursa copulatrix of the female. In Acanthoscelides obtectus the final substances transferred by the male are those which act to stimulate migration of spermatozoa towards the spermatheca; one or more may well be proteins affecting ovogenesis.

D. T. NORTH: I was not aware of the situation you describe for A. obtectus, and find it most interesting. A word of caution should be expressed, however, in that at the present time we are not sure whether the spermatophore contains any sperm at all and, if so, whether it contains only inactive sperm or if not, whether it contains accessory fluid. This is an important point which we hope to elucidate in the near future.

G

ISOTOPE APPLICATIONS: GENETIC

(Session VIII, Part 2)

Chairman: Ø. STRØMNAES
NORWAY

LA SYNTHESE D'ADN DANS LES NOYAUX GEANTS DES INSECTES – MODALITES DE CONTROLE ET STRUCTURES OBSERVEES DANS LA GLANDE SERICIGENE DE Bombyx mori

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Abstract — Résumé

DNA SYNTHESIS IN THE GIANT NUCLEI OF INSECTS – CONTROL MACHINERY AND STRUCTURES OBSERVED IN THE SILK-PRODUCING GLAND OF Bombyx mori. The existence in many insect organs of giant nuclei without visible chromosomes raises the question of possible homologies between the chromatin structures of these nuclei and those of polytene nuclei or common euploid cells. Studies have been made of the nuclei in the silk-producing gland of Bombyx mori. The DNA synthesis is cyclic. During the third stage there are three successive synthesis cycles, which appear to be relatively autonomous in the individual nuclei. For more than 24 hours after moulting, however, synthesis is greatly reduced; moulting factors thus cause synchronization of all the nuclei. This leads to the conclusion that the triggering of a synthesis cycle is controlled by general factors external to the cell. At the end of larval development, DNA synthesis is suspended at the moment when large-scale secretion of silk begins. Evaluation of the pool of endogenic precursors of DNA shows that it is considerably reduced at the end of the DNA synthesis period. The hypothesis proposed is that large-scale synthesis of fibroin requires polarization of the metabolism, hence the depletion of the nucleotide pool and the end of DNA synthesis. DNA synthesis within a single nucleus is to some extent asynchronic. In particular, a well-defined, delayed-synthesis structure visible only in the female seems to be a possible homologue of a sex chromosome. Other asynchronisms are also apparent, though less clearly. Functional studies thus allow the supposition that in the giant nucleus replication units retain an individuality comparable to that of a polytene chromosome. These observations together lead to the conclusion that a nucleus in the silk-producing gland has physiological and structural characteristics similar to those of a polytene nucleus, differing from it essentially in the lesser degree of condensation of its structures.

LA SYNTHESE D'ADN DANS LES NOYAUX GEANTS DES INSECTES – MODALITES DE CONTROLE ET STRUCTURES OBSERVEES DANS LA GLANDE SERICIGENE DE Bombyx mori. L'existence, dans beaucoup d'organes des insectes, de noyaux géants, sans chromosomes visibles, pose la question des homologies possibles entre les structures chromatiniennes de ces noyaux et celles de noyaux polytènes ou de cellules euploïdes banales. Des études ont porté sur les noyaux de la glande séricigène de Bombyx mori. La synthèse d'ADN s'effectue de façon cyclique. Au cours du 3ème âge, on dénombre trois cycles successifs de synthèse. D'un noyau à l'autre, les cycles de synthèse paraissent se dérouler de façon relativement autonome. Cependant après la mue, et durant plus de 24 heures, la synthèse est très réduite, les facteurs de la mue déterminant ainsi une mise à l'unisson de l'ensemble des noyaux. Ces observations obligent à conclure que le déclenchement d'un cycle de synthèse est contrôlé par des facteurs généraux extérieurs à la cellule. A la fin du développement larvaire, la synthèse d'ADN est suspendue au moment où commence la sécrétion massive de soie. L'évaluation du pool des précurseurs endogènes de l'ADN montre que celui-ci est considérablement réduit à la fin de la période de synthèse de l'ADN. L'hypothèse avancée est que la synthèse massive de fibroïne exige une polarisation du métabolisme d'où résulteraient l'appauvrissement du pool des nucléotides et l'arrêt de la synthèse d'ADN.

L'existence, chez les Insectes, d'organes formés de cellules géantes pourvues de noyaux endopolyploïdes pose de nombreux problèmes. Les conséquences de ce mode particulier de la différenciation cellulaire restent encore inconnues pour la plupart. En revanche, nous commençons à entrevoir une partie des mécanismes responsables de cette évolution. Dans les noyaux polytènes, la réplication des chromatides paraît correspondre aux structures et aux processus correspondants d'une cellule normale, tandis qu'ont disparu les évolutions caractéristiques de la prophase ainsi que les éléments cinétiques de la mitose. Cependant, dans la plupart des noyaux géants, toute trace de structure chromosomique est apparemment absente. De sorte que l'on peut se demander si la synthèse d'ADN et sa régulation, sa composition et les structures auxquelles il est attaché, ne répondent pas alors à une forme plus dégradée encore des cycles nucléaires habituels. C'est à ces questions que nous avons tenté d'apporter quelques éléments de réponse.

La figure 1 rappelle un certain nombre de subdivisions que l'on distingue habituellement dans les processus qui gouvernent la synthèse d'ADN. Au sein de chacune de ces divisions interviennent des éléments régulateurs. Et c'est évidemment au niveau de ces éléments qu'il faut chercher la cause du gigantisme cellulaire. Dans une cellule diploïde, ces processus conduisent généralement à la réplication conforme d'un ADN de composition moyenne inchangée. Et l'on peut se demander si les noyaux endopolyploïdes obéissent à cette règle ou si leur structure particulière autoriserait certaines déviations.

Les données que nous avons rassemblées concernent les noyaux de la glande séricigène de *Bombyx mori*. En passant, nous rappellerons quelques données comparatives tirées de la littérature en nous référant le plus souvent possible à des publications comportant une revue bibliographique étendue.

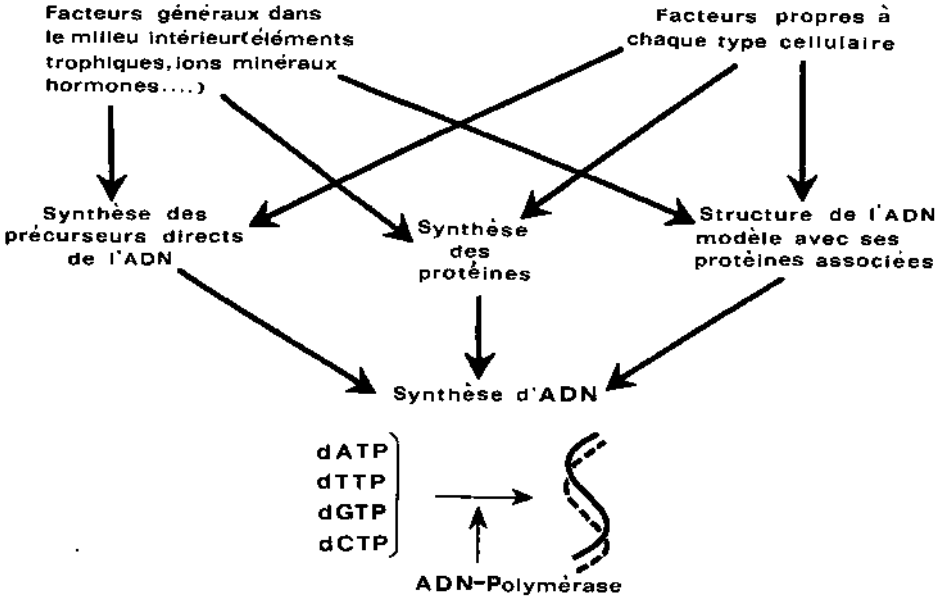

FIG. 1. Schéma simplifié des principaux processus susceptibles d'intervenir dans la régulation de la synthèse d'ADN.

1. LA SYNTHESE DE L'ADN DANS SES RAPPORTS AVEC LA PHYSIOLOGIE DE L'ORGANISME

De nombreuses observations, faites chez les Vertébrés, ont montré qu'une multiplication cellulaire localisée peut être provoquée par des facteurs d'ordre général, hormonal ou autres (1, 2). Pour les Insectes, on citera plus particulièrement deux catégories d'observations :

D'une part, HADORN (3) constate que les cellules des disques imaginaux de Drosophile se multiplient indéfiniment lorsque ceux-ci sont implantés dans l'abdomen d'un imago. Au contraire, leur multiplication s'arrête pour faire place à la différenciation si les mêmes disques sont greffés à la pupe.

D'autre part, KRISHNAKUMARAN et coll. (4) décrivent, dans certains organes des larves de Saturnides, une synchronisation de la synthèse d'ADN par rapport à la mue. Des abdomens de nymphes isolés n'effectuent pas de synthèse d'ADN au niveau de l'hypoderme ; celle-ci reprend lorsqu'on leur greffe des glandes prothoraciques actives. Ces auteurs en concluent que la fourniture d'ecdysone déclenche la synthèse d'ADN dans un certain nombre de tissus.

Dans la glande séricigène de *Bombyx mori*, les noyaux grandissent, sans se diviser et sans jamais former de chromosomes distincts, jusqu'à donner naissance à des structures géantes multilobées, qui peuvent contenir une quantité d'ADN 10^4 fois supérieure à celle d'un noyau diploïde.

Chez des animaux d'âges croissants, le dosage de la quantité totale d'ADN montre que la synthèse répond à un temps de doublement régulier d'environ 43 heures, coupé par des phases de synthèse ralentie, durant 24 à 48 heures, au voisinage de chaque mue (fig. 2) (5). Les résultats de ces analyses sont confirmés par l'étude cytologique. Si on injecte, à un animal, de la thymidine tritiée puis, après un certain intervalle de temps, de la thymidine ^{14}C, on pourra distinguer, parmi les noyaux marqués, ceux qui le sont par le tritium ou par le carbone ou par les deux marqueurs. Lorsqu'on fait varier l'intervalle de temps qui sépare les deux injections, on constate une variation corrélative dans la proportion des marquages mixtes (fig. 3). Sur ce graphique, l'intervalle entre deux maxima représente la durée qui sépare deux phases de synthèse d'ADN au sein d'un même noyau.

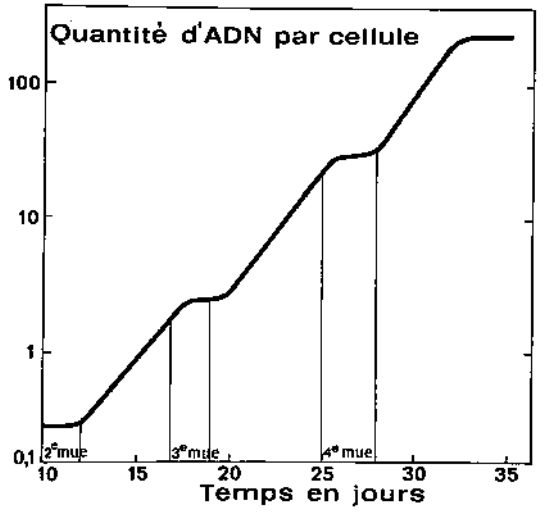

FIG. 2. Evolution de la quantité d'ADN dans la glande séricigène de Vers à soie d'âges croissants. Les temps sont exprimés en jours à partir du premier repas. Les quantités d'ADN sont exprimées en ng ($= 10^{-6}$ mg).

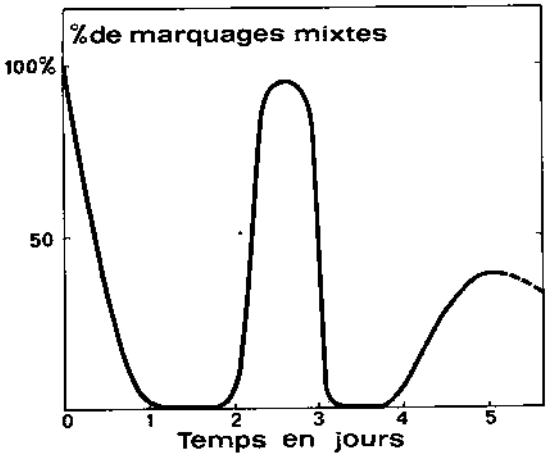

FIG. 3. Evolution du pourcentage de noyaux manifestant un marquage mixte en fonction de l'intervalle entre les deux injections de thymidine ^{3}H et ^{14}C. Vers du troisième âge. La première injection est pratiquée à la sortie de mue.

Appliquée systématiquement aux vers du 3ème âge, cette méthode a démontré l'existence de trois cycles de synthèse : le premier dure 60 heures, tandis que les deux suivants durent moins de 48 heures. Au moment de la mue, le nombre de noyaux marqués par une seule injection tombe aux alentours de 25% alors qu'il peut dépasser 75% un jour plus tard.

Ces observations conduisent à penser que, après la mue, les noyaux mènent à leur fin les synthèses déjà commencées mais suspendent l'entrée dans un nouveau cycle : dans cette hypothèse, la durée prolongée du premier cycle serait la conséquence d'un retard dans le déclenchement de la synthèse.

L'existence de cette discontinuité présente l'avantage évident d'assurer une homogénéisation entre les divers noyaux de la glande : en l'absence de ce processus, en effet, la variabilité individuelle des divers noyaux pourrait introduire des différences croissantes dans leurs développements respectifs. Il paraît évident que cette discontinuité trouve son origine dans un facteur extérieur à la glande. Les observations de KRISHNAKUMARAN inciteraient à en chercher la cause dans un changement de la situation endocrine. Cependant, bien d'autres facteurs peuvent être invoqués, comme des modifications au niveau de métabolites essentiels, tels qu'ils peuvent résulter des processus de la mue associés au jeûne que subit l'animal à ce moment.

2. LA REGULATION DE LA SYNTHESE D'ADN AU NIVEAU DU METABOLISME CELLULAIRE

Lorsque plusieurs noyaux se trouvent dans une même enveloppe cellulaire, on constate en général que leur comportement est étroitement synchronisé. L'une des plus intéressantes expériences effectuées en ce sens a comporté l'utilisation de noyaux prélevés dans le tissu nerveux d'un *Xenopus* adulte et donc présumés incapables de se diviser : lorsqu'ils sont injectés à des oeufs, ces noyaux effectuent une synthèse d'ADN (6). Cette homogénéité du comportement nucléaire ne peut être due qu'à des facteurs intracellulaires.

Parmi les éléments susceptibles d'intervenir à ce niveau, plusieurs auteurs ont noté l'existence d'une corrélation entre l'importance de la synthèse d'ADN et certaines variations au sein du système producteur des nucléotides précurseurs de l'ADN (7). Dans la glande sericigène de *Bombyx*

nous avons pu constater une évolution du pool des précurseurs thymidiliques qui paraît en rapport avec l'intensité de la synthèse d'ADN. Ce pool des précurseurs thymidiliques représente une quantité extrêmement faible, environ mille fois plus petite que la quantité de thymine présente dans l'ADN de la glande. Son évaluation n'a pu être faite que par des méthodes indirectes fondées sur l'étude cinétique des conditions de compétition entre la thymidine radioactive exogène et les précurseurs endogènes, dans des glandes explantées *in vitro* (8).

L'ensemble des mesures effectuées conduit aux résultats du tableau I.

TABLEAU I. SYNTHESE DE L'ADN ET NIVEAU DES PRECURSEURS ENDOGENES
(Les chiffres sont donnés en quantité de thymine (μM) pour 100 mg d'ADN)

Age de la glande étudiée	Incorporation horaire dans la synthèse d'ADN	Quantité présente dans le pool endogène
4ème jour du 5ème âge	20 à 40	10 à 20
6ème jour du 5ème âge	5	1

L'existence de ces variations au niveau du pool des précurseurs est intéressante à plusieurs titres :

1. Elle conduit à considérer avec prudence toutes les évaluations quantitatives de la synthèse d'ADN fondées sur des mesures d'incorporation d'un précurseur radioactif. Chaque fois que des évaluations comparatives portent sur des cellules différentes ou sur des stades éloignés de la vie d'un animal, elles doivent prendre en considération une possible variation au sein du pool des précurseurs endogènes avec lesquels le précurseur radioactif entre en compétition.

2. Les rapports entre ces variations et les changements dans la synthèse d'ADN peuvent être conçus de deux façons au moins. Ou bien on supposera que la réduction du pool des précurseurs est une conséquence indirecte d'une synthèse d'ADN diminuée ; ou bien on admettra que la synthèse réduite d'ADN résulte de l'appauvrissement en précurseurs.

En l'absence d'indications précises, la première hypothèse conserve un caractère spéculatif évident. Au contraire, de nombreuses observations démontrent que si, par l'emploi de moyens génétiques ou physiologiques appropriés, on limite la formation des précurseurs, la synthèse d'ADN se trouve très rapidement bloquée. Dans la glande séricigène de *Bombyx mori*, on peut fort bien supposer que la mobilisation des aptitudes métaboliques de la cellule vers la synthèse massive de fibroïne entraîne des déficiences au niveau d'autres secteurs comme celui qui intéresse la genèse des précurseurs thymidiliques. On touche peut-être alors un aspect particulier de ce problème très général d'un certain degré de contradiction entre la multiplication et la différenciation cellulaire.

3. LE CONTROLE DE LA SYNTHESE D'ADN AU NIVEAU DES STRUCTURES CHROMOSOMIQUES

On sait aujourd'hui que l'ADN d'un noyau est formé par un certain nombre d'unités indépendantes de réplication. Entre ces diverses unités, la synthèse se déroule selon un ordre et avec des durées relatives qui semblent caractéristiques [9, 10]. L'étude des chromosomes polytènes des Diptères est, à cet égard, particulièrement intéressante car elle autorise un pouvoir de résolution très élevé [11, 12]. C'est ainsi que PLAUT et NASH [13] reconnaissent, dans un seul chromosome géant de Drosophile, au minimum 50 unités de réplication distinctes.

Dans les noyaux endopolyploïdes sans structures chromosomiques visibles, l'observation de synthèses asynchrones d'ADN est quasiment inexistante. Une exception remarquable est représentée par le macronucleus du Cilié *Euplotes* où deux ondes de synthèse progressent depuis les extrémités vers le centre du noyau [14, 15, 16].

Chez *Bombyx mori*, le marquage obtenu après incorporation de thymidine radioactive est en général également réparti sur l'ensemble du noyau. Cependant, des marquages limités à certaines parties du noyau ont pu être observés [17]. Ces marquages localisés appartiennent à deux types différents. Les uns présentent la forme d'une tache circulaire dans laquelle la synthèse d'ADN est retardée par rapport au reste du noyau (fig. 4). D'autres au contraire correspondent à des structures épaisses de forme allongée, (fig. 5). La tache circulaire est aisément identifiable et semble correspondre à une structure de la chromatine plus dense que le reste de la chromatine nucléaire. Cette tache est généralement unique : sa taille s'accroît lorsque l'animal grandit ; elle se rencontre seulement chez les individus de sexe femelle. L'ensemble de ces caractères milite en faveur de l'identification de cette structure avec l'hétérochromatine sexuelle : on sait, en effet, que chez le Ver à soie le sexe femelle est hétérogamétique.

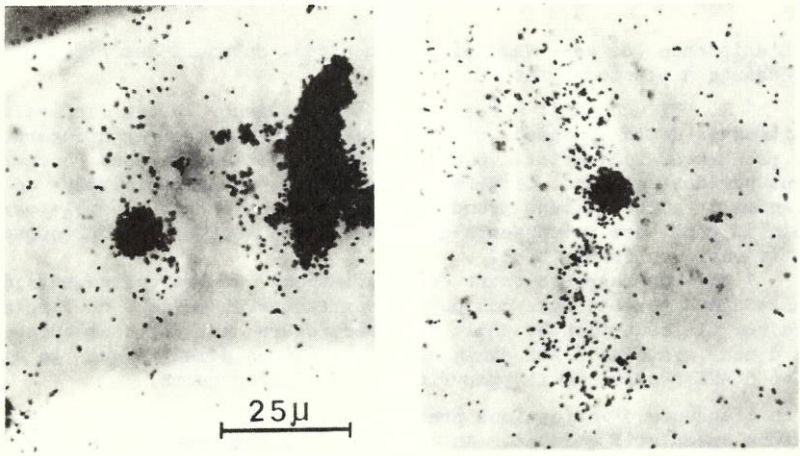

FIG. 4. Marquages nucléaires montrant la tache à incorporation tardive. Sur la figure de gauche, on voit aussi un noyau intégralement marqué. Vers du troisième. âge.

Les structures de forme allongée, en revanche, peuvent être présentes en plusieurs exemplaires au sein d'un même noyau. Mais leurs caractères morphologiques sont trop peu différenciés pour permettre d'établir des homologies précises d'un noyau à un autre. Il semble difficile d'échapper à l'hypothèse que ces structures représentent d'authentiques chromosomes géants.

En définitive, l'emploi de marquages radioactifs permet de reconnaître au sein des noyaux de la glande sécrigène des unités de réplication qui présentent certains caractères de structures chromosomiques gigantesques. Si l'on admet cette interprétation, on est conduit à supposer que l'ensemble du noyau pourrait être formé par l'apposition de telles structures dont la contiguïté interdit normalement de discerner les limites. La forme multilobée du noyau pourrait être une conséquence de l'aspect allongé de ces structures étroitement accollées entre elles. Le nombre de ces éléments correspond-il au nombre des chromosomes ou à un chiffre différent ? Les données actuelles sont encore insuffisantes pour répondre à cette question. On notera seulement qu'une évaluation du volume nucléaire et du volume de ces structures montre que le noyau serait susceptible de contenir entre 20 et 60 éléments de ce genre, tandis que la garniture haploïde du Ver à soie est formée de 28 chromosomes.

4. EXISTE-T-IL UNE SYNTHESE DIFFERENTIELLE D'ADN ?

Malgré l'existence de réplications asynchrones au sein d'un même noyau, on admet le plus souvent que le cycle complet de synthèse corresponde à un doublement rigoureusement conforme de l'ensemble de l'ADN préexistant. Cependant, des observations relativement isolées semblent inviter à la prudence. D'une part, chez la Drosophile, plusieurs auteurs remarquent que la quantité d'ADN hétérochromatique est relativement réduite dans les chromosomes polytènes par rapport à sa proportion dans les cellules euploïdes. D'autre part, des zones de synthèses excessives d'ADN (jusqu'à 15 fois la synthèse normale) ont été notées dans les chromosomes de *Sciara* et *Rhynchosciara*. La question se posait donc de savoir si la structure endopolyploïde n'est pas susceptible de favoriser la synthèse préférentielle de certains secteurs du génome, conduisant éventuellement à un changement dans la composition moyenne de l'ADN. Pour vérifier ce point, nous avons choisi de comparer l'ADN d'embryons à celui extrait de la glande sécrigène. La méthode d'analyse employée a consisté à isoler, dans l'ADN, les divers oligonucléotides pyrimidiques de même longueur, ou *isoplithes*, et à analyser ceux-ci en quantité et en composition (18). Cette méthode fournit, pour un même ADN, un ensemble de résultats qui peut comprendre plus de 15 paramètres d'identification indépendants. Les résultats obtenus montrent que, au degré de sensibilité extrême ainsi obtenu, il n'existe aucune différence significative entre la composition moyenne de l'ADN dans la glande sécrigène et la composition de l'ADN d'embryon. Ainsi, une différenciation cellulaire extrême ne paraît pas, dans ce cas, comporter un changement visible de la composition de l'ADN.

CONCLUSIONS

En définitive, l'ensemble des analyses que nous avons effectuées tend à démontrer que la synthèse de l'ADN obéit, dans les noyaux endopolyploïdes de la glande sécrigène, à la plupart des règles généralement observées dans les cellules diploïdes.

Dans chaque noyau, la synthèse s'opère selon un processus cyclique dont chaque élément correspond sans doute à un doublement. Au sein d'un cycle de synthèse, on découvre des asynchronismes mettant en évidence l'existence d'unités de réplication qui présentent bien des caractères de chromosomes gigantesques. Au cours de la synthèse, la composition de l'ADN semble exactement conservée. Enfin, le déterminisme de la synthèse obéit à divers facteurs dont les uns sont extérieurs à la cellule tandis que d'autres lui sont internes.

FIG. 5. Marquages nucléaires localisés au niveau de formations allongées. Trois coupes successives de la même glande permettent de reconstituer la forme des structures marquées. Les zones claires représentent les sections de noyaux.

L'acquisition la plus importante paraît concerner la nature et la forme des unités de réplication. Bien qu'il soit encore difficile d'exprimer une opinion définitive sur le nombre de ces unités, les éléments recueillis semblent indiquer que les unités observées pourraient correspondre au nombre des chromosomes. Cette situation rappelle fortement les dispositions bien connues au niveau des noyaux polytènes. Elles tendraient à faire penser que, dans le cas de la glande séricigène comme dans celui des noyaux polytènes, les éléments chromosomiques homologues restent associés pour former des unités d'ordre supérieur à comportement synchronisé. Cette situation correspond vraisemblablement à l'absence du processus de condensation caractéristique de la prophase mitotique.

L'une des questions qui se posent est de savoir si cette structure n'est réalisée que dans les noyaux endopolyploïdes de la glande séricigène ou si elle peut exister également dans d'autres organes et dans d'autres espèces. S'il en était ainsi, on trouverait là un élément d'unification entre les processus de différenciation cellulaire particuliers aux Insectes. On notera, seulement, pour terminer, que la prudence apparaît nécessaire avant une généralisation trop étendue. Si l'on considère, en effet, les noyaux polyploïdes des Protozoaires, il apparaît que les observations faites chez *Euplotes* justifieraient peut-être une extension des hypothèses précédentes. Au contraire, les études concernant la régénération du macronucleus chez *Paramecium* ou la division nucléaire chez le Radiolaire *Aulacantha*, conduisent à admettre l'existence de sous-noyaux distincts formés chacun d'un assortiment chromosomique complet. De sorte qu'un ensemble d'observations conduirait à supposer l'existence d'une série d'étapes intermédiaires entre la mitose classique et le processus de multiplication que révèlent les chromosomes polytènes. L'étude comparative de ces divers processus est certainement de nature à nous apporter des éclaircissements importants concernant les relations entre les diverses composantes de la division cellulaire.

REFERENCES

(1) TEIR, H., RYTÖMAA, T., Control of cellular growth in adult organisms. Acad. Press (1967).

(2) LOCKWOOD, D.H., VOYTOVICH, A.E., STOCKDALE, F.E., TOPPER, Y.J., Insulin dependent DNA polymerase and DNA synthesis in mammary epithelial cells in vitro. *Proc. Nat. Acad. Sci.* 58 (1967) 658.

(3) HADORN, E., "Dynamics of determination", in Major problems in developmental biology (LOCKE, M., ed.) Acad. Press (1966) 85.

(4) KRISHNAKUMARAN, A., BERRY, S.J., OBERLANDER, H., SCHNEIDERMAN, H.A., Nucleic acid synthesis during Insect development. II, Control of DNA synthesis in the *Cecropia* silkworm and other Saturniid moths, *J. Insect Physiol.* 13 (1967) 1.

(5) GILLOT, S., DAILLIE, J., Rapport entre la mue et la synthèse d'ADN dans la glande séricigène du Ver à soie, *C.R. Acad. Sc.* (1967) sous presse.

(6) GURDON, J.B., On the origin and persistance of a cytoplasmic state inducing nuclear DNA synthesis in frog's eggs, *Proc. Nat. Acad. Sci.* 58 (1967) 545.

(7) DUSPIVA, F., "Enzymatische Aspekte der Mitose" in Probleme der biologischen Reduplikation (SITTE, P., ed.) Springer, Berlin (1966) 120.

(8) DAILLIE, J., Métabolisme de la thymidine dans la glande séricigène du Ver à soie. I, Les principales voies suivies par le précurseur dans la glande incubée in vitro. II, Utilisation des nucléotides radioactifs pour la synthèse de l'ADN. III, Incorporation dans la glande séricigène prélevée au 6ème jour. IV, Etudes sur la glande in situ. *Ann. Biol. anim. Bioch. Biophys.* 7 (1967) 115 ; 227 ; sous presse.

(9) HSU, T.C., SCHMID, W., STUBBLEFIELD, E., "DNA replication sequences in higher animals" in The role of chromosomes in development (LOCKE M.ed.) Acad. Press (1964) 83.
(10) BARIGOZZI,C., DOLFINI, S., FRACCARO, M.,HALFER,C.,REZZONICO RAIMONDI G. TIEPOLO,L., A first approach to the somatic cell genetics of *Drosophila melanogaster* : studies on DNA replication, *Atti Ass. Genet. Ital.* 12 (1967) 291.
(11) SWIFT, H., "Molecular morphology of chromosome", *In vitro* 1 (1965) 26.
(12) KEYL, H.G., "Lokale DNS-Replikationen in Riesenchromosomen" in Probleme der biologischen Reduplikation (SITTE P., ed) Springer Berlin (1966) 55
(13) PLAUT, W., NASH, D., "Localized DNA synthesis in polytene chromosomes and its implications" in the role of chromosomes in development (LOCKE, M., ed.) Acad. Press (1964) 113.
(14) FAURE-FREMIET, E., ROUILLER, C., GAUCHERY, M., La réorganisation macromoléculaire chez les *Euplotes*. *Exp. Cell Res.* 12 (1957) 135.
(15) GALL,J.G.,Macronuclear duplication in the ciliated protozoon *Euplotes*, *J. bioph. bioch. Cytol.* 5 (1959) 295.
(16) RINGERTZ, N.R., ERICSON, J.L.E., NILSSON, O., Macronuclear chromatin structures in *Euplotes*. *Exp. Cell Res.* 48 (1967) 97.
(17) GILLOT, S., Hétérogénéités fonctionnelles dans l'ADN de noyaux géants. Etude autoradiographique sur la glande séricigène de *Bombyx mori*, *Exp. Cell res.* (sous presse).
(18) NEULAT, M.M., Comparaison de différents DNA animaux par l'analyse de leurs séquences pyrimidiques, *Bioch. Biophys. Acta* 149 (1967) 422.

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