STERILE INSECT TECHNIQUE AND RADIATION IN INSECT CONTROL

PROCEEDINGS OF A SYMPOSIUM
NEUHERBERG
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JOINTLY ORGANIZED BY IAEA AND FAO

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1982

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PROCEEDINGS SERIES

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PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM
ON THE STERILE INSECT TECHNIQUE
AND THE USE OF RADIATION
IN GENETIC INSECT CONTROL,
JOINTLY ORGANIZED BY THE
INTERNATIONAL ATOMIC ENERGY AGENCY
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OF THE UNITED NATIONS,
AND HELD IN NEUHERBERG, 29 JUNE - 3 JULY 1981

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FOREWORD

For the past 25 years, the Sterile Insect Technique (SIT) has been widely investigated against a variety of insect species. Initially these investigations were limited to laboratory and preliminary field studies to determine the SIT's applicability as a pest control measure and to define the requisites necessary for its economic application, whether used alone or in integrated pest management programmes. Today the technique is used widely in various areas of the world to eradicate and/or control insect pests of agricultural and veterinary importance.

To review and discuss this approach to pest control, the Symposium on the Sterile Insect Technique and the Use of Radiation in Genetic Insect Control was jointly convened in Neuherberg, Federal Republic of Germany, by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency, in co-operation with the Gesellschaft für Strahlen- und Umweltforschung mbH, Neuherberg, from 29 June to 3 July 1981. It was an integral part of the IAEA and FAO's effort to promote a greater awareness of the actual and potential application of nuclear techniques to the solution of problems of disease vectors and agricultural pests, and in integrated pest management. The Symposium not only served as a forum for the exchange of research information on the most recent developments in the field, but also as a review of research, development and implementation of investigations conducted to date.

A total of 83 participants and observers from 31 countries and five international organizations attended, 39 papers were presented and six posters exhibited. Four main topics were covered; a review of the SIT against various insect pests; its application to tsetse flies in eradication programmes; quality control of mass-reared insects for release; and the development of genetic approaches to insect mass rearing and control. Other topics emphasized integrated pest management, computer models and radioisotope labelling. A special round table discussion on tsetse fly research with special reference to the IAEA/Federal Republic of Nigeria Co-operative Project on the Biological Control of Tsetse Flies by the SIT was also held. The papers and the summaries of the poster presentations are published in these Proceedings.

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PRESENT STATUS OF THE STERILE INSECT TECHNIQUE

Session 1

Invited Review Paper

PRESENT STATUS AND FUTURE TRENDS OF THE SIT APPROACH TO THE CONTROL OF ARTHROPOD PESTS

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Abstract

PRESENT STATUS AND FUTURE TRENDS OF THE SIT APPROACH TO THE CONTROL OF ARTHROPOD PESTS.

The Sterile Insect Technique (SIT) has slowly developed during the past quarter century as a useful or potentially useful method for the eradication or containment of certain major insect pests, including the screwworm, Mediterranean fruit fly, melon fly, Mexican fruit fly and pink bollworm. The technique has been shown to achieve suppression of several other major pests including the boll weevil, codling moth, tsetse flies, certain mosquitoes, horn fly and stable fly. Steady progress is being made by scientists in various countries on several aspects of the SIT or alternative genetic manipulations that can eventually lead to more extensive and more practical use of the autocidal approach to the management or elimination of major pests. These include: (1) the discovery of various genetic mechanisms that when introduced into natural pest populations will adversely affect the normal development of the pest population; (2) improvements in the mass production of insects both from the standpoint of cost and quality of the reared pests; (3) a better understanding of the ecology, dynamics and behaviour of certain pests that are suitable candidates for practical use of the technique; and (4) a recognition of the advantage of integrating compatible or complementary suppression techniques. The concept of managing populations of major pests on an areawide or ecosystem basis at a strategic time and/or place during seasonal or periodic cycles of pests offers important economic and ecological advantages over the limited crop-to-crop defensive system of management that has been relied upon for decades. The areawide management system designed as a preventive measure can take optimum advantage of the SIT or genetic manipulations that may be of little or no practical value after a pest population has already reached or threatens economic losses. It is likely that the autocidal method will be of most value when appropriately integrated with other compatible suppressive measures. Pests that have heretofore been considered beyond the scope of areawide management because of their mobility and wide host range, such as the *Heliothis* complex, are discussed and the role that autocidal methods could play in their management considered. This pest complex serves as an example of the potential role that autocidal techniques might eventually play in managing many other major pests.

It has been a quarter of a century since the introduction of the Sterile Insect Technique (SIT) as a method of insect pest suppression. Therefore, it is appropriate to make an assessment of the contributions the technique has made in coping with pest problems and consider the role it might play in the future.

The mechanism of pest population suppression by the SIT as described by Knipling [1] has added a new dimension to pest population eradication, containment and management, especially when integrated with other suppressive measures. It is a highly sophisticated method, and its development and practical use involve a number of technical and operational problems. The technique has definite limitations because it is not applicable for most pests. At the same time it has features that could contribute to a better solution for a wide range of important pest problems in an effective economical and ecologically sound manner.

Despite slow progress in its development, the SIT has found an important place in dealing with several major pests. Looking to the future, the principle of pest population regulation by the SIT and other genetic manipulation, such as originally suggested by Serebrovsky [2], offers many opportunities, especially in integrated pest management programmes.

PRESENT STATUS OF SIT

Brief comments on some of the programmes involving the use of sterile insects will indicate the current status of this method of suppression. The screwworm, *Cochliomyia hominivorax* (Coq.), is still the model pest problem involving the release of sterile insects. The successes of several programmes indicate the potential role of the SIT for other major pest problems. The eradication of the screwworm from Curaçao and the southeastern United States of America [3, 4] was followed in 1962 by a suppression programme in the southwestern United States. The initial goal was to eliminate the pest from the normal infested areas in the southwest and maintain eradication by a sterile fly barrier along the Mexican border. The long-range movement of the fly from unsuppressed areas in Mexico [5] showed, however, that an effective barrier was not possible for the scope of the project. Excellent suppression was attained, however, which probably averaged between 95–99% for about 10 years. The programme then tended to become less effective primarily because of a number of a number of changes in livestock management practices and an increase in deer and domestic animal populations. This favoured the development and survival of more native screwworm flies, but the rate of sterile fly releases could not be increased because of budgetary limitations. Deterioration of the fly strains under colonization and certain changes in operational procedures to reduce costs may have contributed to reduced effectiveness [6]. Even so, a high degree of control was maintained during the early 1970s and savings to the livestock industry are estimated to have exceeded $100 million each year.

In 1977, the U.S. Department of Agriculture and the government of Mexico jointly initiated an expanded programme. It was designed to push the pest southward and establish a containment barrier in southern Mexico where the land mass is approximately 150 miles wide as contrasted with 1200 miles or more along the United States-Mexico border. Fly production was increased from about 100 million per week to about 350 million per week. In addition, a new suppression component has been added [7], involving the distribution of an attractant bait composition in areas where native fly densities are too high for rapid and effective suppression with sterile flies alone. New fly strains from areas in Mexico are put into production after one or two years of colonization. The expanded programme has been very successful. At the present time, established populations have been pushed back to approximately 400 miles from the United States-Mexico border.

The screwworm programme has demonstrated the feasibility of managing a wide-ranging pest in an area consisting of several hundred thousand square miles. The returns to the livestock industry have probably amounted to $10 for each dollar invested.

The SIT has also been demonstrated to be effective against several species of tropical fruit flies [8, 9]. It is now accepted as a necessary component for the elimination of Mediterranean fruit fly, *Ceratitis capitata* (Weid.), introductions. The technique was used in 1967 to eliminate a relatively small introduction in the Los Angeles area of California. It is the primary suppressive measure now employed in an effort to eradicate a much larger infestation in California that became well established in the San Jose area in 1980. This infestation, which had spread into an area of about 300 to 400 square miles, reached a high level in an area of about 50 square miles before effective suppressive measures could be started. The programme, which got under way in August 1980, is being conducted by the U.S. Department of Agriculture and the California Department of Food and Agriculture. It has involved the use of bait sprays, fruit stripping and sterile fly releases, but the release of sterile flies is the primary suppression component.

It is difficult to make an assessment of the degree of success of an eradication programme. There is no way of knowing how an introduction would develop in the absence of eradicative measures. However, the summer and fall climate and the kind and amount of host fruits in the infested area seem to be near ideal for medfly development. In my opinion, the population would have grown to a tenfold or higher rate per generation during the summer and early fall months if no suppressive measures had been applied.

The initiation of the programme was handicapped because of restrictions on the use of bait sprays, a proven method of medfly eradication [10]. Also, the number of available sterile flies was limited. Enough flies were furnished, however, by a mass rearing facility in Mexico and by the research facility in Hawaii to release about 20 to 40 million flies weekly during the first several months

of the programme. The number has gradually increased to more than 50 million weekly.

Most of the sterile flies are being released in the area of original establishment, but many sterile flies are also distributed in a surrounding quarantine area of 300–400 square miles. The strategy is to contain spread primarily by the release of sterile flies while suppressing the population in the well-established area by the use of insecticides, sterile fly releases and stripping of fruit. Sprays have been limited, however, to select properties. Probably less than 25% of the native fly population was subjected to insecticide applications. Fruit stripping was not initiated on a large scale until January 1981.

Despite the handicaps in implementing the type and intensity of suppressive measures that should be used under such circumstances, the results indicate that the programme is succeeding. The native fly population based on trap capture data began to level off soon after sterile fly releases were initiated, and then began to decline before the onset of cold weather. There was no evidence of a growing population or an expansion of the infested area as would have been expected if no suppressive measures had been applied.

I estimate that the fly population had reached a level of about 20 000 by the time significant control efforts were undertaken, and that possibly an additional 200 000 potential adult progeny were in various stages of development. Based on a tenfold increase rate per generation, a population of the order of 20 000 adults in August could be expected to increase to a level of 20 million in three generations. Trap capture data indicate that such population growth did not occur. About 200 native flies, primarily males, were captured in Medlure traps. Based on the recapture rate of released sterile flies, it is estimated that about 0.1% to 0.2% of the native flies were taken in the traps. On this basis the accumulative total native fly population may have reached a level ranging between 200 000 to 400 000. During the interim about one billion (one thousand million) sterile flies were released, which would mean an overflooding rate of more than 2000:1.

A few native flies were captured throughout the winter. Approximately 25 female were captured in April 1981, but the number declined in May and June 1981. There is every indication that the programme is succeeding.

An important lesson can be learned from the current eradication programme in California. The regular medfly survey programme was inadequate to detect the infestation before it had grown to a rather high level. Also, provisions must be made in advance for the rapid production of adequate numbers of sterile flies for use in such emergency. The U.S. Department of Agriculture and the interested states plan to maintain a rearing facility in order to ensure adequate numbers of flies for release within a few weeks after the discovery of any new infestations that may occur in the future. While insecticide sprays can be used to eliminate infestations, it is questionable if extensive spraying will be permissible, especially

in residential areas. Therefore, early detection is vital. With authorization to make limited use of sprays in and around centres of new infestations, and with ample sterile flies for the immediate containment of flies in possible areas of spread and to snuff out populations reduced by minimal use of sprays, there is every reason to have confidence that the medfly will not become a permanent pest on the United States mainland.

In my opinion, the current infestation would have got out of hand if the sterile fly technique had not been available. The cost to the U.S. fruit industry would be high if the pest should become firmly established in California and eventually in other states, and the direct and indirect losses would no doubt amount to hundreds of millions of dollars each year. In addition, the need for insecticide sprays to control the pest would seriously jeopardize the integrated insect management programmes on citrus and other fruits in the various states. Growers now rely heavily on natural or introduced biological agents to regulate a wide range of pests with minimal need for insecticides.

The technique is being employed on even a larger scale in a jointly sponsored Mexico-U.S. programme in southern Mexico. P. Patton of Mexico surveys the nature and results of this important eradication and containment programme in a review paper.[1] Other programmes involving the use of SIT for eradication and suppression of tropical fruit flies are dealt with in papers presented at this Symposium. Fruit industries throughout the world are indebted to the many scientists who have contributed to the present status of SIT for coping with tropical fruit fly problems.

The sterility technique is being used to prevent the establishment of another important pest, the pink bollworm, *Pectinophora gossypiella* (Saund.). This major pest of cotton spread from Texas into New Mexico, Arizona, southern California and northwest Mexico during the mid 1960s and threatened the major cotton-growing area in the San Joaquin Valley in California. Although the technology for use of the SIT had not been well developed and demonstrated, this method seemed to be the only feasible and acceptable way to prevent establishment of the pest in the San Joaquin Valley. Therefore, the U.S. Department of Agriculture and the California Department of Food and Agriculture developed a programme, and each year since 1967 sterile moths have been released in the valley. The presence of sterile moths provides a means of inhibiting reproduction of the relatively few moths or their progeny that do reach the valley by flight or other means. The number of pink bollworms released has varied considerably but in most years 100 million or more have been released. Pheromone traps are used for detection and monitoring. The ratio of released males to native males in traps

[1] PATTON, P., "Programa contra la mosca del Mediterráneo en México", these Proceedings, IAEA-SM-255/2.

indicates the magnitude of the overall flooding rate. The trap data generally show sterile to fertile ratios exceeding 1000:1 early in the season. Whereas the ratio in the particular habitats where native moths are present governs the degree of suppression achieved, there is little question that the technique has served its purpose. The dynamics of the pink bollworm in the favourable irrigated environment during the regular growing season is such that a single mated female during the first generation of development would be likely to produce a thousand or more progeny by the fourth generation, which normally represents the overwintering generation. There is little indication that such growth rates have occurred during the 15 years that the programme has been under way.

The probability is very high that the pest would be well established in the San Joaquin Valley if the sterile release programme had not been undertaken. The establishment of the pest in the San Joaquin Valley could cost growers many millions of dollars each year. Also, the IPM programme in this intensive agricultural area would be in serious jeopardy if routine insecticide applications were needed for a key pest like the pink bollworm.[2]

The SIT has been investigated as a method of control for the codling moth, *Laspeyresia pomonella* (L.), in a number of countries. It has been difficult to demonstrate that the technique can be used as a practical alternative to spraying. Research conducted in the state of Washington has been summarized by Huff and White [11]. The general conclusion is that the technique is not practical. The most definitive programme has been undertaken in western Canada under the leadership of M.D. Proverbs, who permitted me to review a manuscript summarizing the results of three seasons of control in a reasonably well-isolated apple-growing area consisting of about 600 acres of apples and pears.

In general, good results were obtained by releasing sterile moths. Some operational problems and severe budgetary constraints were encountered. However, the conclusion is that the technique based on current technology is not fully competitive with spraying.

In my opinion, the most important obstacle to the use of the sterility technique as an alternative to spraying can be attributed to the influence of migrating moths

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[2] In late June 1981, after the foregoing statement was prepared for the Symposium, more than 100 medfly larvae-infested fruit (primarily apricots) were discovered during a two-week period. The larval discoveries were primarily associated with a portion of the infested area where approximately 8 million flies from a single shipment had been released. The timing and distribution of larval discoveries were sufficiently correlated with the release of this shipment of flies so as to suspect that some of these flies were fertile. One released female from among some 300 recaptured flies contained fully developed ova and was considered fertile. The other flies were sterile. Thus the sudden upsurge in infestations may be attributed to the accidental release of some unirradiated flies along with the sterile flies in part of the general release area.

into small experimental areas. As I have stated on numerous occasions, most insects disperse more widely than is generally believed. The movement of relatively few prior-mated females can mean localized concentrations of progeny that reduce the effectiveness of the technique. Less than the expected results in small-scale trials is almost invariably attributed to a failure of the technique rather than the manner the technique is employed. The concept of codling moth management by SIT is sound. The basic technology is well advanced. If a programme were instituted throughout a large or well-isolated codling moth ecosystem, the problem of immigrating moths or their progeny would become minimal. Initially, such a programme may require intensive cultural measures on non-commercial host trees and proper spraying of all orchards until the native moth population in the total codling moth ecosystem is greatly reduced. A greatly reduced population could then be overflooded with relatively few sterile moths. If such a programme were undertaken there is no question in my mind that a large area could within two or three years be managed effectively and at less cost per year by relying primarily on sterile moths than is now required by the use of insecticides alone. A programme based on such a plan would have to be properly organized and executed. Optimum efficiency in large-scale production of moths would have to be attained. The entire area would have to be critically monitored for early detection of incipient infestations originating from long-range migrants or other introductions. Results reported by Proverbs show that in much of the acreage codling moths had been virtually eliminated. The major cost of continuing management would be a thorough monitoring programme and prompt action by whatever methods that would be necessary to deal with threatening infestations.

While some improvements in technology may be required it is my conviction that for the western United States and western Canada where wild host trees or abandoned orchards are limited, the basic technology is adequate to make use of the SIT as the primary system of managing this costly and ecologically undesirable pest. The alternative must also be considered. Unless such an approach is given an adequate trial, we shall have to depend on ecologically disruptive chemicals for the next half century as we have in the past.

Substantial progress has been made in the use of SIT as a supplement to insecticide applications for the suppression of tsetse fly populations. The technique was investigated in Zimbabwe with *Glossina m. morsitans* [12], and subsequently investigated in Tanzania, where more than a quarter million laboratory-reared males were released against a natural population that was reduced by prior applications of insecticides. The flies proved to be highly competitive and maintained suppression at about the 90% level for a number of months in an area that was not well isolated. Similar progress has been made in the Upper Volta with sterile *G.p. gambiensis* [13]. In my opinion, remarkable advances have been made in the development of basic technology, and important biological and ecological information has been obtained that is necessary for eventual practical

application of the technique. The possibility of rearing an insect having such low reproductive potential seemed almost insurmountable less than two decades ago. Important aspects of the biology, ecology and behaviour of these insects has been obtained that is relevant to genetic control, as well as other methods of suppression. A major difficulty in demonstrating practical use of the technique, which is also common to other insects, is the factor of tsetse fly movement. This tends to confuse results and leads to questions regarding the practical application of the technique. However, as for many other major pest species, when appropriately integrated with other methods the technique is sound in principle and desirable from an environmental standpoint. It could play an important role in tsetse fly management programmes in the future.

The release of sterile boll weevils, *Anthonomus grandis* Boh., has been shown to be a useful suppression component when eradication is the objective. A three-year trial eradication programme undertaken in North Carolina and adjacent Virginia was completed in 1980. The nature and results of the programme are in the process of publication [14] and will not be discussed in detail here. The development of the technique for the boll weevil has been difficult. However, co-operative investigations at the U.S. Department of Agriculture Boll Weevil Research Laboratory in Mississippi led to the development of satisfactory rearing and sterilizing procedures. The release of sterile boll weevils is intended for the elimination of very low level populations. Bollweevils can be mass produced at a cost ranging from between $2 to $3 per 1000. When reduced to levels of one or two overwintered boll weevils per acre by prior use of insecticides, it would be more economical to eliminate such greatly reduced population by releasing 1000 or more sterile boll weevils per acre than to continue applying insecticides. This is representative of the manner that the SIT can be used to eliminate other pests. However, since the eradication of most insects is not feasible, more consideration should be given to the role that the technique can play in the organized management of major insect pests. The technology for eradication has been demonstrated and there would be long-range economic and environmental advantages to eradication. However, an alternative to eradication under consideration is to manage rigidly the pest on a year-to-year basis by the use of insecticides after the cotton crop has matured.

A well-conducted management programme based on the use of insecticides would be likely to maintain overwintered populations at an average level of about 10 per acre. It would be economically feasible to release up to 2000 sterile boll weevils per acre to suppress further such low populations. In an optimum boll weevil management programme it is estimated that about four insecticide applications in the fall will be necessary to ensure non-damaging populations during the next growing season. The cost of rearing and releasing 2000 or more sterile boll weevils in the spring should be less than the cost of four insecticide applications in the fall. The final result may be the same. This is an example of a management

system based on the use of sterile or genetically modified insects that offers prospects of being more economical than chemical control. At the same time such a system would involve sound ecological principles for the management of a key pest, which is now lacking in most pest management programmes.

The very brief discussions of several SIT programmes indicate the progress and problems in making use of the technique. Advances have been made on other important insects including the stable fly, *Stomoxys calcitrans* L., the horn fly, *Haematobia irritans* L., and certain mosquitoes. However, it might be said that the technique is serving a vital role in dealing with several important pest problems that cannot be resolved by other acceptable methods. The prospects are excellent that genetic manipulations can eventually play a prominent role in dealing with many other pests in a manner that would be effective and economical; and certainly more sound from an ecological standpoint than to rely primarily on chemical insecticides.

SOME IMPORTANT DEVELOPMENTS RELEVANT TO AUTOCIDAL METHODS OF PEST SUPPRESSION

The use of insects that have been made completely sterile has received most attention in past research. However, insect geniticists and entomologists have made progress on other genetic mechanisms that offer prospects of being much more effective than the conventional sterility procedure. Also, continuing progress has been made in the colonization and mass rearing of insects [15]. Much of the emphasis in the past has been on producing large numbers of insects for release, often without adequate attention to the quality of the insects. There is a better understanding of the biology, dynamics and dispersal behaviour of many pests that are candidates for suppression by autocidal methods. Advances have also been made on other methods of suppression that may eventually be integrated with autocidal techniques. These include outstanding progress on insect pheromones and other behavioural chemicals [16], augmentation of biological agents [17] and more acceptable chemical insecticides. New insect detection methods, by the use of sex pheromones and other attractants, will help to advance autocidal techniques. Concurrent releases of appropriate pest-specific biological organisms and the release of genetically altered insects provide a complementary integrated suppression system that would be ecologically sound and highly efficient.

In addition to new technology, recognition of the benefits that can be gained by employing two or more complementary suppressive measures should eventually contribute to new pest suppression strategies. To appreciate fully the merits of appropriate integrated systems it is important that pest managers understand the mechanism of action of various suppression techniques in relation to the density

and dynamics of a pest, and how the techniques interact when employed concurrently or sequentially. Knipling [18] gave special attention to the advantages that can be gained by integrating various techniques of suppression.

It is not feasible to go into detail in discussing various new developments that are relevant to the management of insects by autocidal means. However, I would like in particular to discuss the hybrid sterility and the inherited sterility mechanisms, which are likely to be much more effective than the conventional method of sterilization.

Laster [19] made crosses of *Heliothis virescens* Fab. and *H. subflexa* (Guenee). Males of the resultant progeny are sterile and the females are fertile. When the F_1 hybrid females are back-crossed to *H. virescens* males the F_2 males continue to be sterile and the females continue to be fertile. Laster subsequently determined that this sterility mechanism persists for an indefinite number of generations.
This male sterility effect was initially suggested as a possible way of producing sterile males that may be more competitive than those sterilized by irradiation; However, Knipling and Klassen [20] and Knipling [18] developed simulation suppression models which indicate that the release of both hybrid sterile males and hybrid fertile females would be potentially more effective than the release of males only. The key to effective suppression by this unique genetic effect is the transmission of sterility in the male progeny by the fertile hybrid females. This provides a new mechanism of suppression that could have great practical significance in the management of *H. virescens* and possibly other pests. *H. virescens* is one of the most important insect pests in the United States of America. The USDA Bioenvironmental Insect Laboratory at Stoneville, Mississippi, and the Mississippi State Experiment Station are collaborating in a pilot programme on the island of St. Croix to evaluate the method. It is under the leadership of F.I. Proshold. The investigations have already demonstrated that the hybrid sterility factor can be introduced into a native moth population, and that it persists for a number of generations. The final effect on the population has not yet been determined, but the results are sufficiently encouraging to indicate that the mechanism offers exceptional promise. The method of calculating the theoretical effect of the hybrid sterility method is involved, and details will not be described here. However, Table I shows the theoretical trend of: (1) an uncontrolled population having an average fivefold increase rate; (2) a population subjected to a 9:1 overflooding rate during the first generation by releasing sterile males that are assumed to be fully competitive; and (3) a 9:1 overflooding rate of hybrid moths, in which the males are sterile and the females are fertile.

The simulation model is based on the assumption that the hybrid males are fully competitive in mating but that hybrid or normal females with which they mate continue to be as attractive as virgins to subsequent matings. Normal and hybrid females mated to normal males are assumed to be only one half as attractive to male as are virgins. A natural mortality of 25% daily is assumed. Males mate

TABLE I. THEORETICAL TREND OF *Heliothis virescens* POPULATIONS SUBJECTED TO:
(1) no control; (2) release of 9000 100% sterile moths of both sexes in generation 1; (3) release of 9000 hybrid moths of both sexes in generation 1[a]

Gen.	(1) Uncontrolled population	(2) 100% sterile moths	(3) Hybrid moths		
			Normal moths	Hybrid moths	Total
1	1 000	1 000	1 000	9 000	10 000
2	5 000	500	500	4 500	5 000
3	25 000	2 500	250	2 250	2 500
4	25 000+	12 500	125	1 125	1 250

[a] Based on parameters and calculations described in Ref. [18].

only one time during a 24-h period but are capable of mating each day. On the basis of these parameters the hybrid males play a primary role in delaying successful matings by normal females. The hybrid females play a primary role in reducing the number of normal X normal matings and in perpetuating the genetic mechanism.

The practical significance of the hybrid sterility effect discovered by Laster cannot be fully assessed at this time. But if the effects come even reasonably close to performing in natural populations as simulation models indicate, and if similar hybrid mechanisms can be discovered for other important Lepidoptera, this genetic mechanism could greatly advance the use of genetic manipulations in the management of these important insect pests.

The type of genetic effect observed in the two Lepidopterous species also occurs among other arthropods. Graham et al. [21] found that when the ticks *Boophilus microplus* and *B. annulatus* (Canestrini) are crossed the male progeny is sterile and the female progeny is fertile. R.L. Osburn (unpublished data) determined that the sterility effect persists in the male progeny for three back-crosses and then partial fertility is restored. Osburn and Knipling [22] considered the possibility of making use of hybrid ticks as a supplemental suppression method in *Boophilus* tick eradication programmes. The results of theoretical calculations will appear in Ref. [22]. However, Table II shows the assumed trend of an uncontrolled tick population starting from a low level, and the trend of a similar population subjected to the release of enough hybrid larvae to result in a 5:1 ratio of hybrid-to-native adult ticks on the host animals. The possibility of

TABLE II. THEORETICAL TREND OF AN UNCONTROLLED *Boophilus* TICK POPULATION VERSUS A POPULATION SUBJECTED TO THE RELEASE OF ENOUGH HYBRID LARVAE EACH PERIOD TO RESULT IN AN AVERAGE RATIO OF APPROXIMATELY FIVE ADULT HYBRID TICKS TO ONE ADULT NATIVE TICK ON EACH INFESTED HOST ANIMAL[a]

		Uncontrolled population		Controlled population	
3 months period	Host population	Total adult ticks (native) (No.)	Hosts infested (%)	Total adult ticks (native) (No.)	Hosts infested (%)
1	1 000	10 000	50	10 000	50
2	1 000	20 000	100	3 332	17
3	1 000	40 000	100	976	5
4	1 000	80 000	100	312	1.6
5	1 000	160 000	100	100	0.5
6	1 000	320 000	100	32	0.2
7	1 000	640 000	100	8	0

[a] Summary of theoretical results reported by Osburn and Knipling [22].

adding hybrid tick larvae directly on host animals in lieu of dipping is also suggested. The hybrid sterility mechanism may well provide a new and important component to *Boophilus* tick eradication programmes in various parts of the world. If so, this could make a major contribution to a more successful way of coping with one of the world's most important parasitic disease problems.

Another genetic mechanism that remains to be fully exploited is the inherited genetic damage resulting from substerilizing dosages of irradiation. It has been known for a number of years that males of Lepidoptera receiving partial sterilizing dosages of irradiation (5–15 krad) produce F_1 progeny that has a higher level of sterility and other detrimental effects than the parent male.[3] North [23] reviewed the rather extensive investigations on low-level irradiation. Most of the research on the genetic effects produced by low dosages of irradiation involved back-crosses with normal insects. Generally, the effect on inbred crosses was not determined. The effects involving back-crosses rapidly diminish after the F_1 generation in most species. Consequently, the full potential of the low-dosage irradiation technique

[3] 1 rad = 1.00×10^{-2} Gy.

was not revealed. However, Walker and Quintana [24] reported the collapse of laboratory populations of the sugar-cane borer, *Diatraea saccharalis* Fab., when male parents received low substerilizing dosages. Nielsen and Brister [25] demonstrated persistent genetic effects in the greater wax moth *Galleria mellonella* (L.) through five generations of back-crosses. Amoako-Atta and co-workers [26] and Brower [27] showed that substantial genetic effects persist through the F_3 generation when males of the almond moth, *Cadra cautella* (Walk.), received low irradiation dosages. Brower [28] obtained similar results with the Indian meal moth, *Plodia interpunctella* Hubner.

The effect on the dynamics of a pest population resulting from low-level irradiation cannot be readily visualized by merely considering the laboratory data. Also, the influence of low-level irradiation when descendants of irradiated parents are back-crossed to normal insects is not necessarily the most important effect. Reduced survival of progeny resulting from matings between descendants of the original treated parent or parents may be of greater significance.

It is necessary to establish simulation models in order to make an appraisal of the potential effect that can result by releasing moths receiving low levels of irradiation. Simulation models must take into account the types of matings that will occur in successive generations and the degree of survival of progeny, as observed in laboratory crosses.

I have made use of the laboratory results reported by Nielsen and Brister [25] to calculate the theoretical effect of moths receiving low-dosage irradiation when introduced into normal populations. The results are given in the publication referred to. I have also used data published by Amoako-Atta and co-workers and Brower to calculate the theoretical effect on the dynamics of treated populations when overflooded with moths receiving low irradiation dosages. Details of the calculations will not be given here, but the procedure used is described in Ref. [25]. Table III shows the theoretical impact on a lepidopterous pest population subjected to the release of male moths receiving 10 krad irradiation, based on results reported by Amoako-Atta and co-workers [26]. The assumed release rate is 9:1. The natural population is assumed to have a fivefold increase rate. The theoretical trend of an uncontrolled population and a population subjected to a 9:1 ratio of completely sterile moths is shown for comparison.

The calculations indicate that the ultimate effect of 10-krad males released at a 9:1 ratio would be much greater than the release of the same number of completely sterile moths. It would be necessary to release more than five times as many completely sterile moths during the initial generation to equal the effect produced by the moths receiving the low dosage. This does not even take into account that moths receiving 10 krad are likely to be more competitive than those 100% sterile.

The simulation model is based on the release of males only. If both males and females are released the theoretical effect is even more favourable for the low-dosage method.

TABLE III. THEORETICAL TRENDS OF LEPIDOPTEROUS PEST POPULATIONS SUBJECTED TO:
(1) no control; (2) release of 9:1 ratio of 100% sterile males during generation 1; (3) release of 9:1 ratio of 10-krad males during generation 1

Gen.	(1) No control		(2) 100% sterile males		(3) 10-krad Males[a] Normal		Descendants of 10-krad males	
	♂	♀	♂	♀	♂	♀	♂	♀
1	1 000	1 000	1 000	1 000	1 000	1 000		
2	5 000	5 000	500	500	500	500	3 847	1 283
3	25 000	25 000	2 500	2 500	290	290	1 025	362
4	25 000	25 000	12 500	12 500	320	320	1 211	851

[a] Based on survival data from various matings in successive generations kindly furnished by B. Amoako-Atta, from Ref. [26].

The low dosage technique unquestionably involves both dominant and recessive genetic action. The dominant effect is expressed in back-crosses to normal insects. The recessive effect is expressed when descendants of the original treated parent or parents interbreed. Scientists developing genetic manipulations are faced with a dilemma in efforts to evaluate fully and demonstrate the practical utility of such complex mechanis. The effect on fitness for survival is difficult to determine beyond the F_2 generation even in the laboratory because of the increasing number of mating combinations that are subsequently involved. Field experiments involving a range of dosages and ratios of treated-to-normal moths are virtually out of the question. Research on laboratory populations is the only feasible way to obtain basic data, and simulation models based on such data are almost essential to estimate the possible effect of releases against natural populations. The only practical procedure will be to conduct appropriate pilot field experiments based on laboratory data and simulation models. Enough basic information has already been obtained on several species of moths to indicate that a major investment in further laboratory studies and in appropriate field experiments is fully justified to determine the potential of substerilizing dosages of irradiation for the management of important lepidopterous pests, and possibly other insects.

Other genetic manipulations are under investigation that have not been discussed. The opportunities for the future to deal with insects of agricultural and

health importance are almost limitless. It is my feeling that basic knowledge and technology already available are 10 to 15 years ahead of a good understanding and acceptance of the way genetic manipulations could be used in insect management systems. The economic and ecological advantages of this method of insect pest management are so great that the science of entomology may fail to take full advantage of a powerful and ecologically desirable pest regulating mechanism if it does not fully exploit the potential that genetic methods hold for the future.

AREAWIDE PEST MANAGEMENT CONCEPTS

The trend of thinking by many entomologists is in the direction of organized areawide management of major pests. The present IPM system is basically a defensive method relying on year-to-year and crop-to-crop control of pests as the need arises. Such a defensive system will tend to force growers or health officials to continue to rely on fast-acting insecticides to deal with the pests when they threaten a crop or create a health problem. Unfortunately, most alternative control methods under development will not be effective or practical after the pests have already reached damaging or threatening levels. The methods include various genetic manipulations, sex pheromones, augmentation with slow-acting parasites, predators or pathogens, growing host plant resistant varieties, or various combinations of these methods. Important contributions have been made during the past two decades on various techniques that could contribute to preventive entomology. Also, much basic information on the ecology and dynamics of many pests has been obtained which is relevant to areawide management approaches. However, these important developments will remain largely dormant, and may never be put to practical use unless leaders in the entomological community recognize the advantages and support areawide preventive management concepts for some of the more damaging and formidable pests. The implementation of areawide pest management programmes will require special financing and fully qualified personnel to plan and execute such programmes. Although many problems remain to be resolved, the economic and ecological advantages to society are so great that the science of entomology can hardly afford not to develop fully area-wide pest management strategies for major pests that take full advantage of suppression techniques which are ecologically sound, instead of relying primarily on ecologically disruptive insecticides.

POSSIBILITIES FOR AREAWIDE MANAGEMENT OF *Heliothis* spp.

Several dozen major insect pests that occur in the United States of America or other parts of the world could be selected as examples to show the potential

advantages of the areawide population management approach. Various techniques or combination of techniques might be considered to achieve such an objective. However, I would like to analyse what is generally acknowledged as the most important insect problem in the United States of America and discuss the potential advantages of the areawide preventive management procedure versus the year-to-year and-crop-to-crop management procedure that is now practised. I refer to the two *Heliothis* species, *H. zea* and *H. virescens.* Since my topic deals with autocidal techniques I will indicate how this method could be a major component in the areawide management approach. However, other techniques may eventually prove to be equally or more practical.

At the end of 1980 I participated in a meeting with several authorities on the *Heliothis* complex. The meeting was held on 15 December 1980, and was attended by D.F. Martin, E.G. King, E.A. Stadelbacher and J.W. Smith of the U.S. Department of Agriculture Laboratory at Stoneville, Mississippi, and by C. Lincoln, J.R. Phillips, G.W. Barnes and A.J. Mueller of the University of Arkansas. The purpose of the meeting was to consider the following question I had posed: Would it be advantageous economically and ecologically to manage *Heliothis* spp. on an areawide basis by attacking populations in an organized manner during generation 1, as opposed to the prevailing practice of managing the pests during generations 3 and 4 on a farm-to-farm and crop-to crop basis when economic treatment levels occur?

Details of the meeting will be discussed elsewhere, but I would like to summarize the general conclusions gained from an analysis of the question.

The two species of *Heliothis* attack a wide range of crops. Losses are estimated to average more than one billion dollars per year [29]. Many biological agents help regulate their abundance but self-perpetuating populations do not provide dependable control. Insecticides, either chemical or biological, are required for control on cotton, tobacco, sweet corn and other crops after populations reach damaging levels. The present control procedures rely on close monitoring of populations to determine if and when the benefits of insecticide applications justify the cost of control. Much effort for years has been devoted to the development of crops resistant to *Heliothis* attack. While this is a highly desirable method, it is very unlikely that suitable varieties will be developed and widely accepted for all crops affected. The chemical insecticides used on cotton and other crops cause severe ecological disruptions, and once started treatments are generally necessary until the crop matures. The crop-to-crop approach to *Heliothis* management has been practised for decades and may have to continue indefinitely because alternative methods now envisioned are not likely to be practical and effective after populations have already reached damaging levels.

Despite their wide distribution, mobility and abundance, it has been my view for years that *Heliothis* management procedures for use on an areawide or even on a regional or national basis before the pests reach damaging levels could be

TABLE IV. THE DYNAMICS OF A STEADY DENSITY *Heliothis* POPULATION CONSIDERED REPRESENTATIVE OF THE ANNUAL CYCLE IN MISSISSIPPI OR ARKANSAS

Heliothis generation	Adult population (estimated)	Host plant acreage	Moths per acre	Eggs per acre	Estimated increase rate
1[a]	16 500 000	250 000	66	10 000	3
2	50 000 000	3 500 000	15	3 750	4
3	200 000 000	2 000 000	100	25 000	6
4	1 200 000 000	5 000 000	240	60 000	0.9
5[b]	1 080 000 000	–	–	–	–

[a] Generation 1 would be overwintered survivors.
[b] Potential adult progeny which overwinter as diapausing larvae. A survival of 1.6% of the potential overwintered population is assumed.

developed, and would be the most logical solution for these major pests. The use of *Heliothis* specific methods would be emphasized to conserve the natural biological control agents that exert strong regulatory action but which often fail to provide satisfactory control, especially in our greatly altered agro-environments. Autocidal methods, resistant host plants when available, augmentation with *Heliothis* specific biological control agents, *Heliothis* specific insecticides, and the use of sex pheromones or appropriate combinations of such methods would meet the requirements for ecologically sound approaches to *Heliothis* management. In my view, present IPM programmes, which by definition are intended to be based on sound ecological principles, will not meet the definition so long as broad spectrum insecticides are required as the major component in an integrated programme. For the reasons stated, I feel that the preventive approach making maximum use of ecologically sound techniques will be the most rational approach to the management of the *Heliothis* complex as well as many other important insect pests. The rationale for preventive entomology seems equally as sound as preventive medicine.

In an effort to analyse the question previously stated, a population model was first developed that is typical of the seasonal trends of *Heliothis* populations in states such as Arkansas and Mississippi. The model, which admittedly needs refinement and validation as more definitive information becomes available, is shown in Table IV.

The model reveals three major features that support the rationale for areawide management of *Heliothis* during the early part of the season, rather than delaying suppressive measures until populations increase to high levels and invade a wide range of crops.

The overwintered *Heliothis* generation is largely concentrated on wild host plants. The total acreage of wild hosts is estimated to be less than 10% of the acreage of crops subject to damage by the 3rd and 4th generations. This feature supports the logic of applying suppressive measures at this strategic time and place by making use of several techniques that may not be practical or feasible on more extensive crop acreages. A second feature revealed by the model is the great expansion of the *Heliothis* habitat as the season advances, which means that there will be a comparable expansion of the habitat for the natural biological agents. It is small wonder that natural biological control agents cannot be counted on to regulate effectively *Heliothis* populations. The great and rapid expansion of the *Heliothis* host plant acreage means that there will be a consistent dilution of natural biological agents per unit of host-searching area year after year. This factor, together with the lag time for an adequate buildup of biological agents, explains why natural control agents are not likely to prevent the development of potentially damaging *Heliothis* populations on a wide range of crops in intensive farming areas. The third feature of the model that is particularly relevant to the subject of this Symposium is the relatively low number of moths that normally survive the winter. If the estimated population of 16 500 000 *Heliothis* moths is even close to normal numbers, the use of the autocidal technique would seem to offer a practical and feasible method for areawide management. Based on the estimated density, it seems appropriate to consider the economics of *Heliothis* management by autocidal means as compared with current practices, which rely primarily on insecticide applications later in the season. The participants of the meeting referred to above estimate that an average of $35 million is spent by growers in Arkansas and in Mississippi for *Heliothis* control, principally on cotton. In addition, however, it was estimated that losses will average $15 million yearly, despite the cost of control. Thus, a figure of $50 million can be regarded as the annual damage in each state.

If the inherited hybrid sterility mechanism for *H. virescens* and/or the inherited genetic effects due to low irradiation dosages could be introduced into the natural population during generation 1 or generations 1 and 2, it is possible that most of the damage from *Heliothis* would be avoided on most major crops for the entire season. The study group estimated that 90% suppression of reproduction may accomplish this objective. Based on theoretical calculations, such level of suppression might be achieved or even greatly exceeded by an effective ratio of 9 released to 1 native moth during generation 1. This ratio based on the natural density estimate would require the production and release of about 150 million moths. If we assume a cost of $10 per 1000 moths produced and

released in very large-scale operations, the cost would be $1.5 million to accomplish areawide management. This would not include the costs of facilities, monitoring, administration, etc., that would be necessary for any pest management system. This cost should be compared with the $35 million now spent on chemical control measures. The theoretical effects obtained in models are not likely to be realized in actual practice. However, if the actual cost of autocidal control were five times above the estimate, this technique could still save growers in Mississippi or Arkansas millions of dollars each year. If projected on a regional basis the savings to the agricultural economy could amount to several hundred million each year. However, this is not the only potential benefit that should be considered. Adequate management of *Heliothis* by autocidal means would eliminate the current need for ecologically disruptive chemicals for *Heliothis* management. The development and use of insect pest management procedures that cause minimal environmental problems has been and should continue to be a major goal of scientists engaged in pest management activities.

The conference considered other approaches to areawide management of *Heliothis* that may be equally as practical and desirable as the autocidal technique. These included certain cultural measures, application of *Heliothis* specific pathogens, release of *Heliothis* specific parasites, use of *Heliothis* pheromones by confusion or mass trapping procedures, or various combinations of such techniques. However, I believe that the model depicted in Table IV clearly indicates the justification for full exploration of the autocidal method.

Whereas genetic methods offer excellent prospects for achieving areawide management of *Heliothis* populations, the point that I wish to emphasize is the concept of areawide management of total populations to maintain populations below levels of significant crop losses, regardless of the techniques employed.

GENERAL COMMENTS

The *Heliothis* complex was selected as a prime example of the potential economic and ecological advantages of the management of pest populations in an organized manner as a preventive measure rather than to deal with segments of populations in an unco-ordinated manner on specific crops if and when economic threshold levels are reached. The potential advantage of the total pest population management approach could apply for a wide range of insects. These may include but not be limited to such consistently damaging crop pests as the boll weevil, pink bollworm, codling moth, fall armyworm, *Spodoptera frugiperda* J.E. Smith, European corn borer *Ostrinia nubilalis* (Hubner) and cabbage looper, *Trichoplusia ni* (Hubner). This approach could be equally as advantageous for a number of major livestock pests including *Hypoderma* spp., stable fly, housefly, *musca domestica* (L.), face fly, *musca autumnalis* DeGeer, and horn fly.

It may seem highly presumptuous, premature and unrealistic to propose that the areawide management concept would be applicable for such a wide range of pests. However, I would urge pest management authorities to analyse critically all of the major pest problems from the standpoint of possible long-range management strategies taking into account available or envisioned techniques of management. The techniques that might be applicable for areawide management should be considered in depth, and the possible long-range economic and ecological advantages of areawide versus the farm-to-farm management procedure should be considered. Depending on the nature of the pest, areawide management may make effective use of the augmentation of biological agents, genetic manipulations, cultural measures, conventional or promising new insecticides, new attractants or various combinations of techniques used at strategic times and places. The crop-to-crop management procedure as the need arises cannot take advantage of most of these techniques. I am confident that a critical and comprehensive analysis of all major pests, taking into consideration the technology that has been or which might be developed, would support areawide population management as a logical solution for many of the most damaging and ecologically disruptive insect pests in various parts of the world. Whereas various techniques may be employed to advantage for such an approach, I am confident that genetic manipulations can play a prominent role in future pest management systems. Symposia such as this can well encourage a continuation of research to develop further this promising and ecologically desirable method of insect pest suppression.

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Memoria general encargada

PROGRAMA CONTRA LA MOSCA DEL MEDITERRANEO EN MEXICO

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Abstract–Resumen

THE MEDFLY PROGRAMME IN MEXICO.

The Mediterranean fruit fly was detected in Mexico for the first time in January 1977 in the area of Soconusco in Chiapas State adjoining Guatemala. After this first detection Mexico activated the preventive programme which was started in 1927, when external quarantine No.5 was promulgated and the first sampling systems were put into effect. The activities were reinforced by the experience gained in Guatemala through the Joint Mexican-Guatemalan Commission formed in 1975. Subsequently, in 1977, immediate assistance was received from the IAEA, FAO and the Regional International Organization of Plant Protection and Animal Health, and under an agreement with the United States Department of Agriculture. The offensive phase of the programme began with the following aims: stopping the advance of the pest towards the north of Mexico, eradication in Mexico and Guatemala and, in the long term, elimination in Central America. The invasion of the medfly in Mexico, favoured by the characteristic environmental conditions of the area and abundance of hosts, was impressive: during only two cycles the populations increased by a factor of 50 and spread to 3.5 million hectares of Mexican territory. In 1979, when the full complement of trained technical personnel, materials, equipment and facilities were available, the process of eradication went into action. The medfly programme in Mexico has been successful in that it has checked the movement of the pest towards the north of the country, reduced the populations to very low levels and eradicated the pest from more than 3 million hectares.

PROGRAMA CONTRA LA MOSCA DEL MEDITERRANEO EN MEXICO.

La mosca del Mediterráneo fue detectada por primera vez en México en enero de 1977, en la zona del Soconusco del Estado de Chiapas, colindante con Guatemala. A partir de esa primera detección del insecto, se activó el programa preventivo establecido en México desde 1927, año en que se promulgó la cuarentena exterior N°5 y se aplicaron los primeros sistemas de muestreo. Asimismo se reforzaron las acciones con la experiencia adquirida en Guatemala a través de la Comisión Mixta México-Guatemala formada en 1975. Posteriormente, en 1977, se contó con el apoyo inmediato del OIEA, de la FAO, del Organismo Internacional Regional de Sanidad Agropecuaria y con un convenio suscrito con el Departamento de Agricultura de Estados Unidos. El programa comenzó su fase ofensiva estableciendo como objetivos: detención del avance de la plaga hacia el norte de México, erradicación en México y Guatemala y, a largo plazo, eliminación de Centro América. La invasión de la mosca del Mediterráneo en México, favorecida por las condiciones ambientales características de la

zona y la amplia disponibilidad de hospederos fue impresionante; en sólo dos ciclos las poblaciones aumentaron 50 veces y se propagaron por 3,5 millones de hectáreas de territorio mexicano. En 1979, al contar con elementos técnicos capacitados, materiales, equipo e instalaciones en forma integrada, comenzó el proceso de erradicación. El Programa Mosca del Mediterráneo en México ha sido exitoso al no permitir el movimiento de la plaga al norte del país, haber reducido las poblaciones a niveles sumamente bajos y al haberlas erradicado de más de 3 millones de hectáreas.

1. PROGRAMA CONTRA LA MOSCA DEL MEDITERRANEO EN MEXICO

Después de una dispersión vertiginosa a través de El Salvador y Guatemala (540 km en línea recta), la mosca del Mediterráneo fue detectada en territorio mexicano en enero de 1977, y las poblaciones de la plaga fueron incrementando en número y en territorio infestado. Las medidas de control tomadas han cambiado ese panorama, teniendo a la fecha un programa exitoso.

Aunque en forma inmediata y emergente se emprendieron acciones tanto de tipo cuarentenario como de aspersión de insecticida con atrayente, fue a mediados de 1979 cuando se intensificaron de manera decisiva los sistemas de combate. En 12 meses (de julio de 1979 a junio de 1980) se asperjaron por vía aérea 350 000 hectáreas en forma repetitiva, totalizando 2 650 000 ha a razón de 1 litro por hectárea (200 cm^3 de malathion ULV (volumen ultra bajo) y 800 cm^3 de proteína hidrolizada). Por otro lado, en julio de 1979, después de un año y medio de iniciarse la construcción del Laboratorio de Cría y Esterilización de Mosca del Mediterráneo, se comenzaron las liberaciones de insectos estériles. Estos dos factores junto con el apoyo de un intenso trampeo, muestreo de frutos y cuarentenas marcaron el cambio de 3,5 millones de hectáreas infestadas en 1979 a 350 000 hectáreas en 1981, declarándose erradicada la plaga de 3,1 millones de hectáreas; colateralmente, trabajando en el norte de Guatemala, se han erradicado brotes que potencialmente podrían invadir la Península de Yucatán en México. En Los Angeles, Estados Unidos, las moscas producidas en el laboratorio de Metapa favorecieron la erradicación de ese brote; hoy día, junio de 1981, se envían 120 millones de pupas por semana.

1.1. Trampeo

Se han utilizado trampas tipo Jackson a razón de 1 cada 2 km^2 aproximadamente. Se ha considerado un promedio de eficiencia de trampeo del 0,05%, tomado como base para determinar el número de moscas nativas, dispersión de moscas estériles y evaluación de las moscas estériles producidas.

El Cuadro I registra el total de detecciones de mosca del Mediterráneo en los últimos 5 años. Esta información, expresada en moscas por trampa y por

CUADRO I. DETECCIONES DE ADULTOS DE MOSCA DEL MEDITERRANEO EN MEXICO

Mes \ Año	1977	1978	1979	1980	1981
Enero	1	32	410	29	3
Febrero	10	74	839	35	5
Marzo	7	62	1406	173	28
Abril	11	54	2784	47	42
Mayo	12	33	952	24	14
Junio	15	50	156	0	
Julio	46	6	10	0	
Agosto	14	1	7	1	
Septiembre	3	1	0	0	
Octubre	2	0	0	0	
Noviembre	0	0	0	0	
Diciembre	0	21	6	0	
Totales	121	334	6570	309	92

día y multiplicado por 10 000 (Fig.1) en el mismo período, muestra claramente la disminución de la plaga en México; los datos para 1981 abarcan los primeros seis meses y se estima que los valores disminuirán al finalizar el presente año.

El Cuadro II muestra la distancia de la detección más lejana con respecto a la frontera entre México y Guatemala. Es importante señalar que, aún siendo en el mismo Municipio de Pijijiapan, en los años 1977, 1978 y 1981 no se ha repetido el mismo sitio de captura, concluyéndose que no es una población establecida.

La Fig.2, dividida en cuadrantes de 10 × 10 km con fines de organización, indica los brotes de adultos en 1981 hasta el 31 de mayo; las detecciones se cifran en un promedio de 1,5 moscas fértiles por brote.

Comparando las detecciones de moscas entre 1980 y 1981 (Fig.3), las capturas en números absolutos han disminuído en 1981. En efecto, en 1980 el promedio de detecciones fue de 1,9 moscas fértiles por brote, mientras que en 1981 ha sido de 1,5, lo que indica una disminución en la infestación. Las aseveraciones anteriores expresadas en el mes de junio obedecen a que en la zona de trabajo las lluvias se establecen en el mes de mayo, disminuyendo casi totalmente las poblaciones nativas.

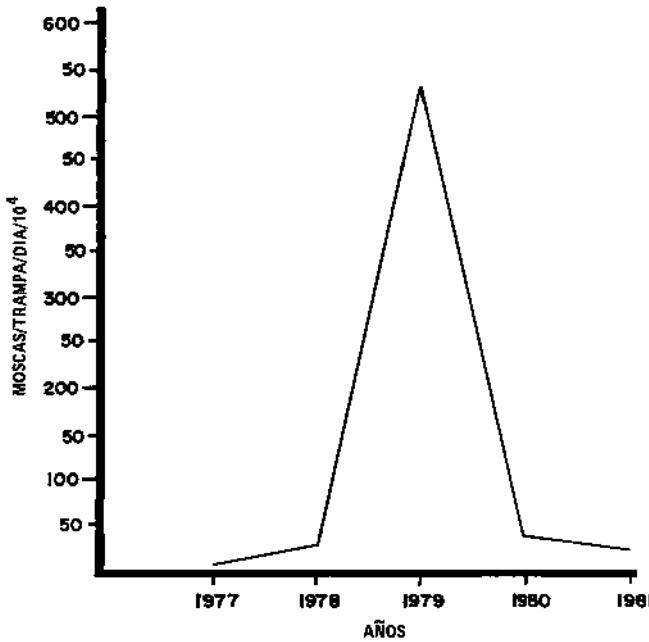

FIG. 1. Fluctuación del MTD de moscas fértiles acumulado desde 1977 hasta 1981.

CUADRO II. DETECCIONES MAS AVANZADAS EN TERRITORIO MEXICANO DE MOSCA FERTIL, EN 5 AÑOS

Fecha	Municipio	Distancia de la frontera con Guatemala (en km)
9 de junio de 1977	Pijijiapan, Chiapas	120
2 de febrero de 1978	Pijijiapan, Chiapas	130
19 de marzo de 1979	Tapanatepec, Oaxaca	220
21 de enero de 1980	Villa Comaltitlan, Chiapas	50
20 de marzo de 1981	Pijijiapan, Chiapas	120

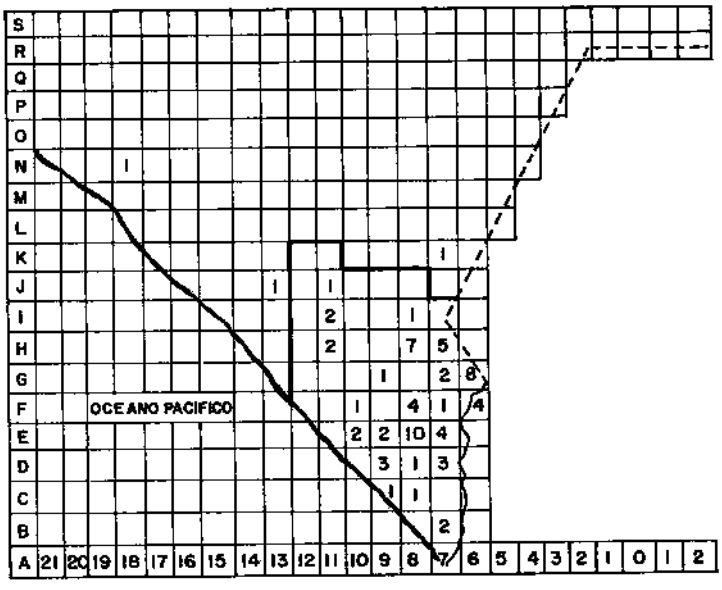

FIG.2. Brotes de adultos por cuadrante – 1981 (hasta el 31 de mayo).

1.2. Muestreo de frutos

La detección de larvas en México comenzó en 1979. En base a las experiencias en Centro América y el resto del mundo, en 1980 se comenzó a muestrear en forma dirigida y se procuró, en la medida de lo posible, seguir patrones estadísticos. Los frutos de caimito son indicativos de la infestación y se consideran hospederos trampa, por lo que en 1980 se inició la compra de ellos.

Hasta mayo de 1981, se encontraron en México los siguientes frutos infestados por la mosca del Mediterráneo:

Café *(Coffea arabiga* (L.)*)*
Naranja agria *(Citrus aurantium* (Osb.)*)*
Naranja dulce *(Citrus sinensis* (L.)*)*
Caimito *(Chrysophyllum cainito* (L.)*)*
Mandarina *(Citrus reticulata* (Blanco)*)*
Chicozapote *(Achras zapota* (L.)*)*
Guayaba *(Psidium guajava* (L.)*)*
Albaricoque *(Micropholis* sp.*)*
Pomelo *(Citrus grandis* (Obsbeck)*)*
Pomarosa *(Eugenia jambos* (L.)*)*

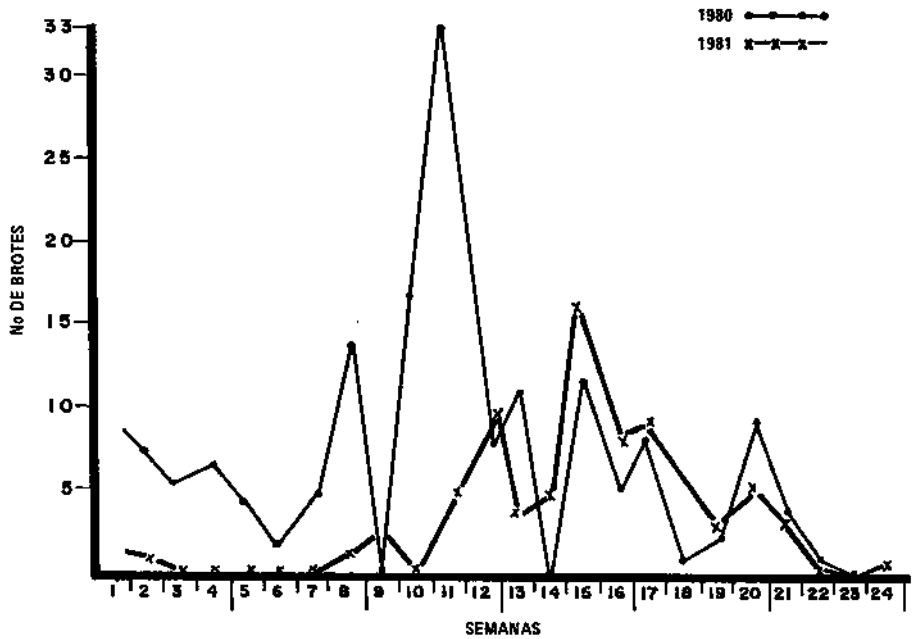

FIG.3. Fluctuación semanal de los brotes de adultos fértiles (1980–1981).

La Fig.4 indica los kg de fruta muestreada por período en 1980 y 1981. El esfuerzo consentido en 1981 ha sido significativo y ha permitido orientar de manera más precisa las actividades de control. Como se observa en la Fig.5, las infestaciones se concentran a lo largo de la frontera con Guatemala.

1.3. Aspersión atrayente – insecticida

Después de la aspersión aérea "masiva" y "dirigida" que se realizó en 1979 y 1980, no fue necesario proseguir con esa intensidad. El trabajo realizado fue sin duda altamente efectivo. Desde julio de 1980 a junio de 1981, las aplicaciones de producto químico fueron exclusivamente terrestres, asperjando de 3 a 5 manchas de mezcla (0,5 ltr de malathion, 5 ltr de proteína, 10 cm^3 de trimedlure y 95 ltr de agua) por árbol; la aspersión promedio fue de 70 000 manchas por semana alrededor de los brotes, en ocho ocasiones como mínimo y con una semana de intervalo.

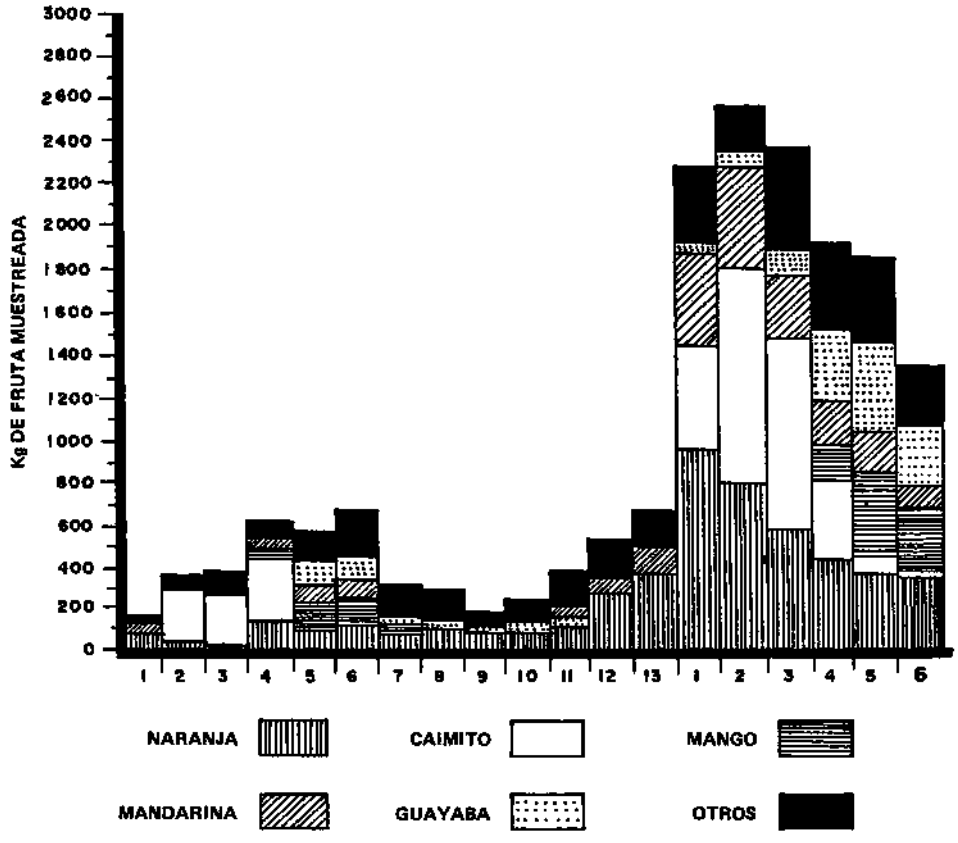

FIG.4. Cantidad de fruta infestada por período y la fluctuación de las 5 frutas más muestreadas durante 1980–1981.

2. LABORATORIO DE CRIA Y ESTERILIZACION

A partir de la primera liberación en julio de 1979 y tras un año (julio de 1980) se logró por primera vez la meta fijada al laboratorio, es decir una producción de 500 millones de pupas por semana; después, hasta 1981, se consiguió superar esa cifra. En 1981, en las primeras 25 semanas se produjeron 13 683 millones de pupas, promediando 547 millones por semana, con un máximo de 771 millones en la semana 15. El sistema de cría empleado es el desarrollado por el Dr. David Nadel en los laboratorios del OIEA en Seibersdorf, Austria, modificado desde luego a las condiciones en México; estas modificaciones han sido, son y serán constantes, hasta el punto de haber tomado el nombre de "Sistema Metapa".

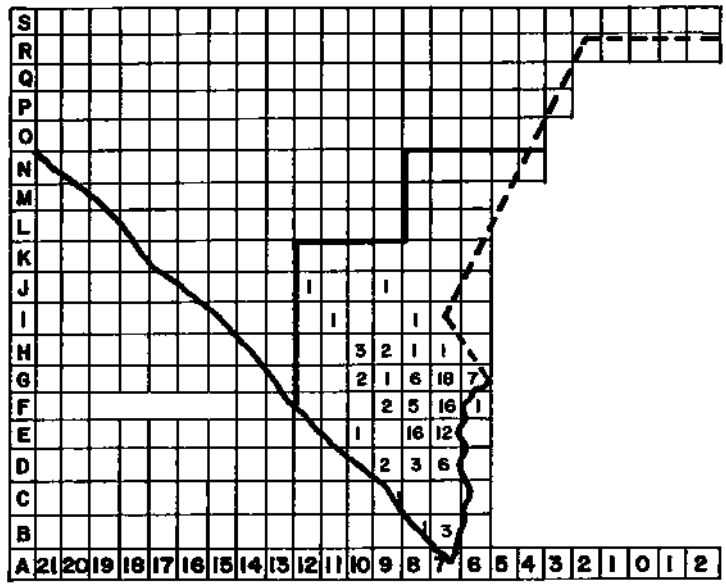

FIG.5. Brotes de larvas por cuadrante – 1981 (hasta el 31 de mayo).

El pie de cría inicial transportado de San José, Costa Rica, a Metapa, en abril de 1979, ha sido criado en cautiverio durante 40 generaciones.

La producción de huevecillos se ha mantenido a niveles superiores a la capacidad de producción (Fig.6). Se cuenta con 76 jaulas en operación, produciendo cada una de ellas un promedio de 33 millones de huevecillos en 12 días. Cada unidad tiene 700 000 moscas en trabajo de reproducción, 8 kg de alimento por jaula (3 partes de azúcar por una de proteína hidrolizada) y agua en abundancia. El sistema rotativo mantiene una producción constante. Una de las metas del laboratorio es sincronizar la producción, con objeto de manejar de la manera más eficiente posible las liberaciones. Los huevecillos son colectados dos veces diarias con 12 horas de intervalo. La maduración de los huevecillos se realiza en botellones con capacidad de 8 ltr; se colocan 250 ml de huevos con 5 ltr de agua, y se inyecta aire comprimido y purificado en forma uniforme a todos los botellones durante 56 horas, tiempo suficiente para obtener el 60% de eclosión.

La dieta para larvas consiste en levadura, azúcar, salvado de trigo, bagazo de remolacha azucarera, ácido cítrico, benzoato de sodio y agua, que se prepara en mezcladoras con capacidad para una tonelada. En 1981 se ha mezclado un promedio de 6,5 ton por día. La dieta se introduce al laboratorio de producción por gusano sin fin.

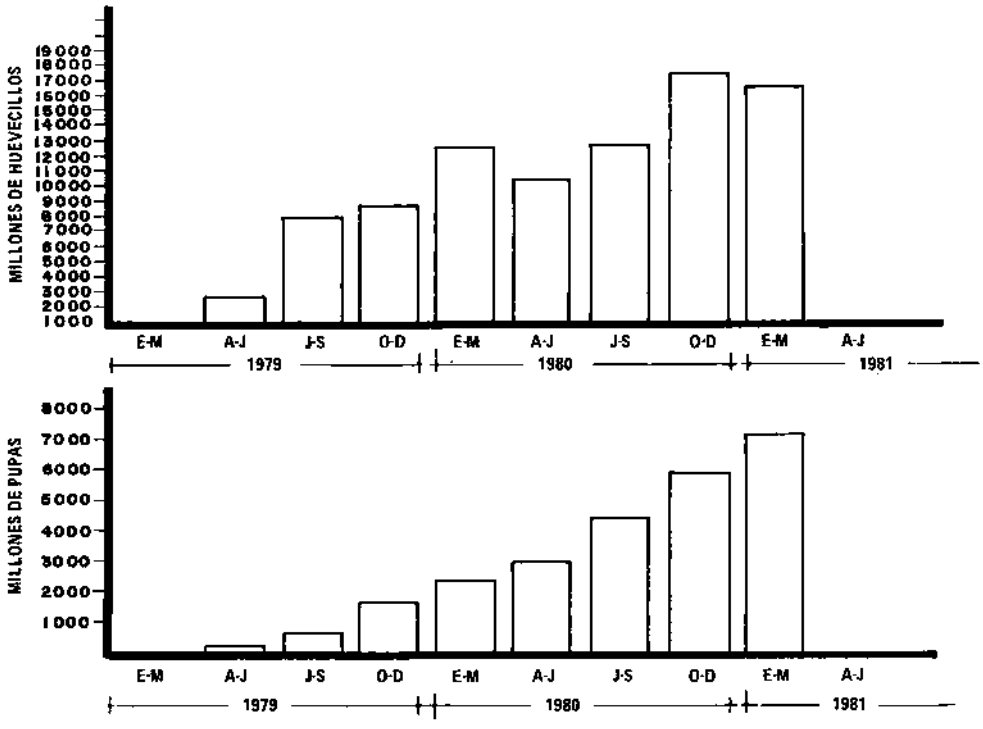

FIG.6. Producción trimestral de huevecillos y pupas.

El laboratorio, al estar localizado en México, requiere seguridad absoluta, no permitiendo el escape de moscas fértiles; existe un único acceso y salida de personal en la planta, y las pupas, dieta y basura pasan obligatoriamente por la fuente de irradiación antes de su salida. Al pasar la dieta de la zona no cuarentenada a una cuarentenada, se vacía ésta sobre charolas a razón de 5 kg por contenedor; diariamente se siembran 1200 charolas con 125 000 larvas cada una (25 larvas por gramo). Las charolas se colocan sobre estantes y se transportan al cuarto de iniciación, donde permanecen 36 h a 32°C y 95% de humedad. Dentro de la sala de larvas pasan cuatro y medio días a 27°C y 70% de humedad relativa, hasta completar ese estadio y ser separadas en las tómbolas. A cada charola se le mezclan 2 kg de salvado de trigo para secar la dieta; se vacían 200 kg en cada tómbola, ésta rota 1/3 de giro cada 5 min y al cabo de 4 horas se han separado más del 95% de las larvas. Se colocan cribas con 2 ltr de larvas cada una en estantes y se transportan al cuarto obscuro, en donde puparán en 24 horas a una temperatura de 24°C y 60% de humedad relativa. Al segundo día de pupación pasan al cuarto de maduración de pupas, donde a 24°C y 60%

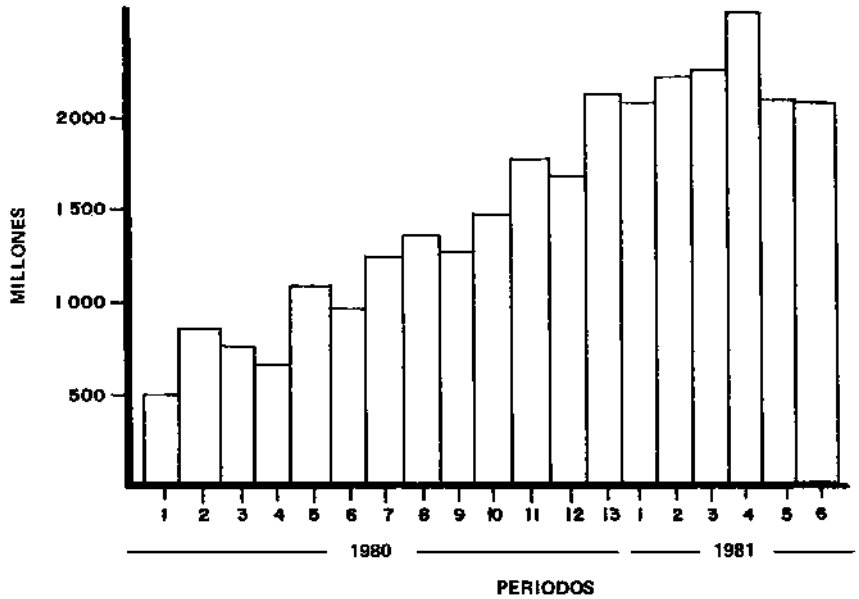

FIG. 7. Producción de pupa fértil por período.

de humedad relativa pasan 6 días. La Fig.7 muestra la producción de pupa a través de los 13 períodos de 1980 y los primeros 6 períodos de 1981.

Una vez constatada la maduración de la pupa, observando la coloración de ojos, se procede al tinte con colorante fluorescente y su empaque en botellones de 12 ltr, tratando de realizar esta operación a 24°C; el cierre es hermético, creando una anoxia por un mínimo de una hora, y se pasa al proceso de irradiación.

La máquina adquirida para irradiar es un JS-7400 de la Atomic Energy of Canada Ltd, con capacidad sobrante para esterilizar tanto pupas como el material de desecho. Por medio de cajas que se mueven sobre una banda transportadora, se expone la pupa a una dósis de 14,500 rad. El manejo de datos se realiza en forma constante, desde la producción de huevecillos hasta su liberación o, en el caso de envíos a otros países, hasta el momento del embarque.

La fase de dispersión comienza al recoger con camiones refrigerados los botellones con pupas estériles en Metapa. Como parte importante del programa, se envían un promedio de 100 millones de pupas por semana a Guatemala (Fig.8) y 122 millones de pupas por semana a California, Estados Unidos (Fig.9).

Durante todo el proceso se realizan rutinariamente 20 pruebas de control de calidad, desde las del tamaño de huevecillos hasta las de orientación al habitat

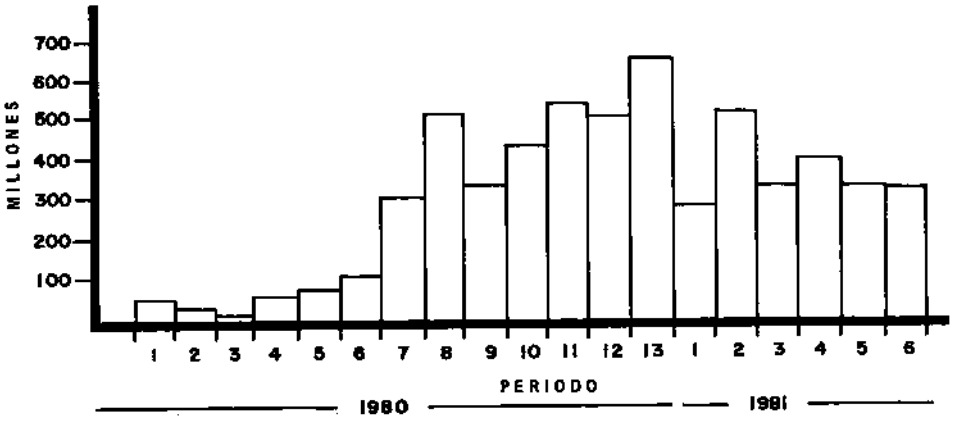

FIG.8. Pupa enviada a Guatemala.

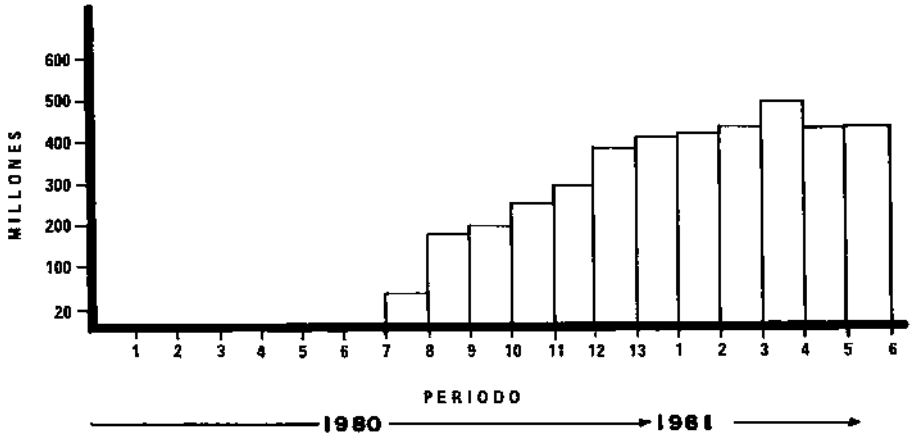

FIG.9. Pupa enviada a Estados Unidos.

en el campo; colateralmente, cada mes se ejecutan 5 pruebas de campo y laboratorio y anualmente se realizan pruebas de comportamiento, índice de aislamiento de moscas silvestres (como complemento, en breve se establecerán las pruebas de olfactometría, electrorretinogramas y otras). Como parámetros generales, la Fig.10 muestra el porcentaje de emergencia e índice de vuelo-preirradiación; puede observarse que se ha logrado mantener un estándar superior a las medias consideradas para la producción. La calidad post-irradiación disminuye un 5% en promedio. El haber alcanzado las metas establecidas se debe en gran medida al hecho de haber realizado 114 trabajos

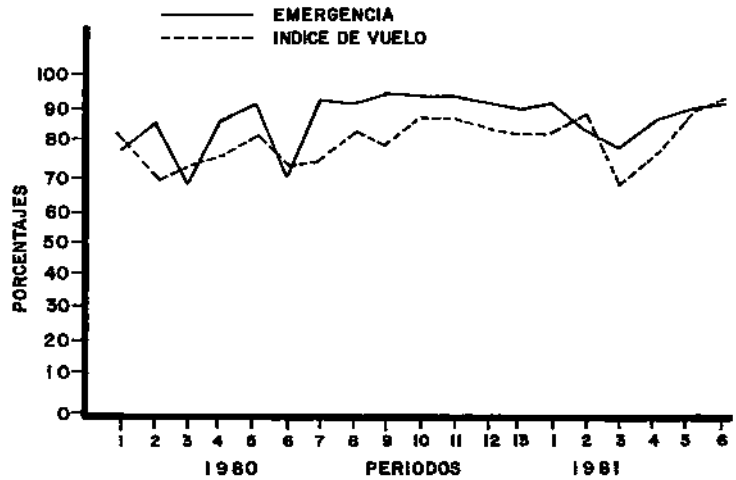

FIG.10. Figura comparativa del porcentaje de emergencia e índice de vuelo pre-irradiación.

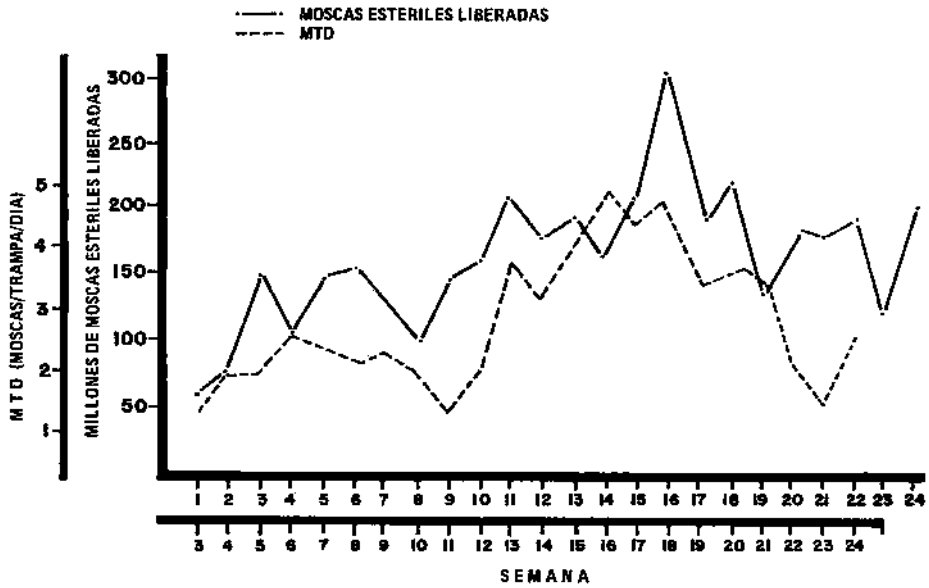

FIG.11. Liberación y recaptura de moscas estériles.

de investigación y mantener la flexibilidad suficiente para implementarlos al resultar positivos.

La liberación, en junio de 1981, se realiza en bolsas de papel, en las cuales se colocan 20 000 pupas, trozos de papel y azúcar; se esperan dos y medio días y se liberan. La recuperación promedio es del 55% de pupa de adulto viable en campo. En un principio, se intentó liberar pupa obteniendo recuperaciones del 12%; posteriormente se utilizó el sistema de adulto frío, logrando recuperaciones de 45%, aunado este a un sin número de problemas mecánicos y técnicos. En un futuro cercano se realizarán pruebas con un nuevo sistema, el de cajas, y si los resultados muestran mejor o igual recuperación que con las bolsas, se adoptará éste.

La recuperación de moscas estériles en las trampas ha sido un indicador supervisor excelente de las actividades. La Fig.11 muestra, mediante moscas por trampa y por día y con datos de 3 semanas posteriores, la recaptura en 1981; en la semana 21 se observa el efecto de un fuerte temporal en la zona, sin embargo la tendencia general es similar a la de las liberaciones.

Considerando el tipo de terreno en que se tendría que trabajar, la abundancia de hospederos, la falta de vías de comunicación y la realidad social imperante, la única alternativa para lograr las metas programadas fue utilizar la técnica del insecto estéril. Además de las 2 unidades técnicas (Operación y Producción), el apoyo administrativo, de mantenimiento y divulgación fueron indispensables para el funcionamiento del programa.

La decisión política y su continuidad han sido factores esenciales para el logro de los primeros objetivos (evitar la dispersión de la plaga al norte de México y erradicarla de México). La erradicación en México se refleja en los datos de junio de 1980 a enero de 1981. Las infestaciones encontradas de febrero a junio de 1981 se considera son movimientos de Guatemala a México, ya sea por humanos o por el vuelo de la mosca. El objetivo a alcanzar ahora es la erradicación del insecto en Guatemala.

Invited Review Paper

THE JAPAN AND TAIWAN PROJECTS ON THE CONTROL AND/OR ERADICATION OF FRUIT FLIES

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Abstract

THE JAPAN AND TAIWAN PROJECTS ON THE CONTROL AND/OR ERADICATION OF FRUIT FLIES.

The fruit flies of economic importance in the far east are the melon fly, *Dacus cucurbitae*, and the oriental fruit fly, *Dacus dorsalis*. The present northern limit of distribution of these flies are the southwestern islands which include Okinawa and Kagoshima Prefecture of Japan. In the Ogasawara (Bonin) Islands in Japan, only the oriental fruit fly lives. For the eradication of the oriental fruit fly, the male annihilation method was used in the Kagoshima, Okinawa and Ogasawara Projects. In the Kagoshima and Okinawa Projects, the eradication could be achieved if a sufficient dose of chemicals was used. However, re-invasion of the flies should be considered even in the situation of islands in the sea. In the Ogasawara Project, the male annihilation method was not successful for eradication, but not because of an insufficient dose of chemicals. Under such circumstances, the application of SIT appeared to be effective. For the eradication of the melon fly, SIT was applied in the Okinawa Project. Estimation of the population density and the calculation of the number of released flies required for achieving the eradication should be performed before releasing the sterile flies. In the experimental release of sterile melon flies in Kume Island of Okinawa, a sufficient number of flies released enabled a successful eradication to be achieved. However, re-invasion from the surrounding islands occurred twice. In Taiwan, SIT and the male annihilation method for the oriental fruit fly have been carried out as a control measure for the integrated control project of the fly. These methods reduced the infestation level of the citrus and mango fruits. From the angle of cost/benefit, these methods were preferable to insecticide spraying.

INTRODUCTION

The fruit flies of economic importance in the fast east are the melon fly, *Dacus cucurbitae*, and the oriental fruit fly, *Dacus dorsalis*. The present northern limit of the distribution of the melon fly is the Amami Islands, and that of the oriental fruit fly is Okinawa Island and the Ogasawara (Bonin) Islands (Fig.1).

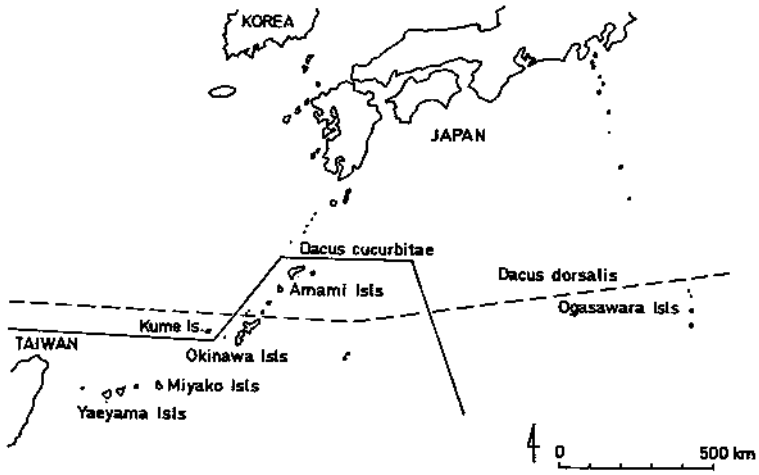

FIG.1. Northern limit of distribution of the oriental fruit fly (– – –) and the melon fly (——) in the far east in 1981.

TABLE I. CURRENT STATE OF THE JAPAN AND TAIWAN PROJECTS FOR THE CONTROL AND/OR ERADICATION OF FRUIT FLIES

Project	Region (area)	Species	Method	Period	Eradication[a]
Kagoshima	Amami Isls (1238.3 km^2)	Oriental fruit fly *(Dacus dorsalis)*	MA[b]	1968–80	+
		Melon fly *(Dacus cucurbitae)*	SIT[c]	1979–	–
Ogasawara	Ogasawara Isls (73.0 km^2)	Oriental fruit fly	MA	1975–76	–
		Oriental fruit fly	SIT	1976–	±
Okinawa	Okinawa Isls (1434.0 km^2)	Oriental fruit fly	MA	1977–	–
		Melon fly	SIT	1972–	±
Taiwan	Taiwan (35 961 km^2)	Oriental fruit fly	MA, SIT	1975–	–

[a] Eradication was achieved (+), partially achieved (±) or not achieved (–) by 1981.
[b] MA: Male annihilation method with poisoned methyl eugenol.
[c] SIT: Sterile insect technique.

TABLE II. DURATION OF APPLICATION OF THE MALE ANNIHILATION METHOD IN THE AMAMI ISLANDS[a]

Name of island	Area (km^2)	Period
Kikai-jima	55.7	1968–79
Amami-oshima	819.1	1973–79
Tokuno-shima	248.1	1972–79
Okierabu-jima	94.5	1970–80
Yoron-jima	20.8	1970–80

[a] Data from Ref. [2].

As there are no fruit flies in mainland Japan, the transport of host fruits of the flies is prohibited from these regions. There have been three projects, Kagoshima, Ogasawara and Okinawa, aimed at eradicating the fruit flies and removing the ban on the transport of host fruits such as citrus and cucurbit. These projects are being carried out by the prefectural governments of Kagoshima, Tokyo and Okinawa, with the financial support of the Japanese national government.

Both the melon fly and the oriental fruit fly can be found in Taiwan, and the oriental fruit fly in particular is economically important. The Joint Commission of Rural Reconstruction and the Provincial Department of Agriculture and Forestry began a field control project for the oriental fruit fly in 1975. The current state of these projects is shown in Table I. The progress of and problems connected with these projects are reviewed.

KAGOSHIMA PROJECT

Oriental fruit fly eradication by the male annihilation method

The oriental fruit fly was first recorded in Kikai-jima in the Amami Islands in 1929, and by 1946 it had invaded all the Amami Islands. To eradicate the flies, the male annihilation method [1] with poisoned methyl eugenol was adopted experimentally in Kikai-jima in 1968. This method was subsequently applied to other islands (Table II) [2].

Methyl eugenol and insecticides were incorporated into various materials such as fibre block, cotton rope and rolled cotton, and dropped by a helicopter on

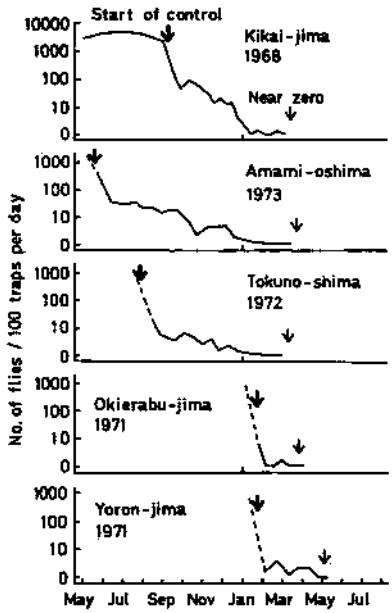

FIG.2. Trends in the number of adult flies caught by monitor traps during the early period of application of the male annihilation method in the Amami Islands [2].

the forests and fields or applied on the ground in the populated areas. The materials, dose of chemicals and frequency of application were changed from year to year. In general, the dose of chemicals was at first large, then reduced after the population density of the flies decreased.

The number of adult flies caught in Steiner-type monitor traps decreased remarkably shortly after the beginning of the control, and practically no flies were recorded within a year (Fig. 2). However, a considerable number of flies were caught in the successive years and eradication could not be achieved before 1979 (Table III). To overcome this situation, the dose of chemicals was increased in 1974 but without any appreciable effect.

Some experiments indicated that the oriental fruit fly inhabiting the Amami Islands did not become resistant to methyl eugenol and/or insecticides. Re-introduction of the fly from the Okinawa Islands was suspected for the following reasons: (1) The adult flies were usually caught in the summer when the wind blows mainly from the south. (2) The number of flies caught was larger in the southern islands, which are close to Okinawa Island where the oriental fruit fly occurred abundantly (Table III) [3]. (3) This species has been known to have the ability to fly at least 50 km over the sea [4]. (4) the re-infestation occurred concomitantly in several islands in the same year, particularly in 1974 and 1977 (Table III).

TABLE III. ANNUAL NUMBER OF ORIENTAL FRUIT FLIES CAUGHT PER 100 TRAPS IN EACH OF THE AMAMI ISLANDS[a]

Year	Kikai-jima	Amami-oshima	Tokuno-shima	Okierabu-jima	Yoron-jima
1968	Start				
1969	10				
1970	22			Start	Start
1971	728			98	135
1972	93		Start	157	730
1973	6	Start	44	216	1235
1974	281	54	346	870	4623
1975	12	1	15	61	625
1976	0	0	3	40	150
1977	22	35	1	19	230
1978	0	0	0	0	5
1979	12	3	5	0	0
Distance from Okinawa Island (km)	220	170	107	57	22

[a] Data from Ref. [2].

This assumption of re-infestation was corroborated by the fact that the number of catches decreased drastically after 1977 when the control of the fly in Okinawa Island began (Table III). In 1980, the eradication of the fly was thought to be complete on the basis of both trap catches and fruit inspection.

Melon fly eradication by SIT

The melon fly, first discovered in Yoron-jima and Okierabu-jima in 1973, immediately expanded its distribution range to all the Amami Islands in 1974. A control project by the sterile insect technique (SIT) aimed at eradicating the melon fly within 10 years from the Amami Islands was begun in 1979 [2].

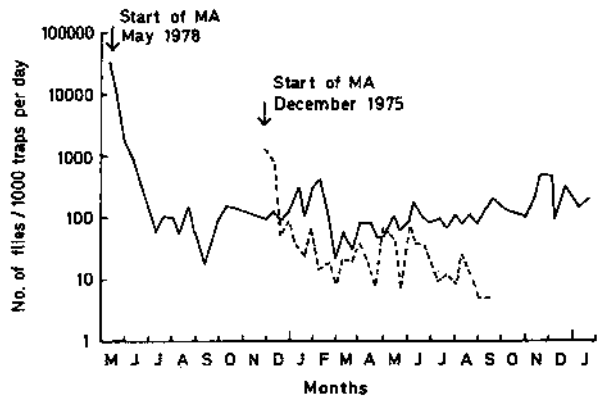

FIG.3. Trend in the number of the oriental fruit flies caught by monitor traps in Chichi-jima in the Ogasawara Islands after the application of male annihilation methods (data from N. Habu).

OGASAWARA PROJECT

Oriental fruit fly eradication by the male annihilation method and SIT

The oriental fruit fly was first recorded in the Ogasawara Islands in 1925. When these islands were occupied by the United States of America, U.S. military forces applied experimentally the male annihilation method to the flies from 1960 to 1962, but the control measures were discontinued before achieving any success. After the reversion of these islands to Japan, the eradication project was undertaken by the Tokyo metropolitan government in 1975 [5].

From December 1975 to September 1976, cotton rope saturated with methyl eugenol and insecticides was dispensed by a helicopter on forests and fields. In the populated areas, fibre blocks were distributed by hand. The number of flies caught in monitor traps was reduced to about 1/100 of the level recorded before the control within three months, but thereafter no reduction was observed (Fig. 3). In November 1976, the release of sterile oriental fruit flies was initiated. About 30 million sterile flies were released up to April 1978. However, the eradication of the flies was not accomplished (Table IV).

In May 1978, a third attempt to apply the male annihilation using cotton rope (spread on forests and fields by helicopter) and fibre block (distributed in populated areas by hand) saturated with poisoned methyl eugenol was made, except in Muko-jima. The dose of chemicals was increased compared with the previous trials. The number of flies caught in monitor traps significantly decreased for about four

TABLE IV. DURATION OF APPLICATIONS OF THE MALE ANNIHILATION METHOD AND SIT IN THE OGASAWARA ISLANDS[a]

Name of island	Area (km^2)	Period	
		MA	SIT
Muko-jima	6.6	1975–76	1976–78, 1978–
Chichi-jima	38.9	1975–76, 1978–80	1976–78
Haha-jima	27.5	1975–76, 1978	1976–78, 1978–

[a] Prepared from N. Habu's personal data.

months after the beginning of the control (Fig. 3). But the decrease was slower than in the Amami Islands (Fig. 2), and the population was maintained at a level of 1/100 compared with that before the control for more than one year (Fig. 3). This situation seemed to be the same as in the previous control. From this fact, it seemed possible that a strain resistant to methyl eugenol had been developed in the Ogasawara Islands. The sterile insect technique has been applied again in Muko-jima and Haha-jima since 1978 (Table IV).

In 1978, the population density of the oriental fruit fly was estimated and the number of sterile flies required to achieve a successful eradication was calculated. About 2–3 million sterile pupae per month were released on Muko-jima from May 1978. In September 1978, the ratio of sterile : wild flies reached about 300 : 1, and the percentage of infestation of host fruit became zero. Such zero level of infestation was maintained for more than one year and it was thought that the oriental fruit fly in Muko-jima had been eradicated.

Since November 1978, 1.5–2.0 million sterile flies have been released weekly on Haha-jima, after calculating the number of sterile flies required for the eradication. Sterile pupae were released by hand on the ground in populated areas, but in forests they were released from a helicopter with a special container. The ratio of sterile : wild flies has been about 30 : 1 since November 1979, and the percentage of infestation of host fruit became zero in September 1980 and has been maintained at such a level subsequently.

In Chichi-jima, the distribution of poisoned methyl eugenol was stopped in June 1980, to prevent an increase in resistance in the populations. Control with poisoned methyl eugenol was to begin again several months before the release of sterile flies.

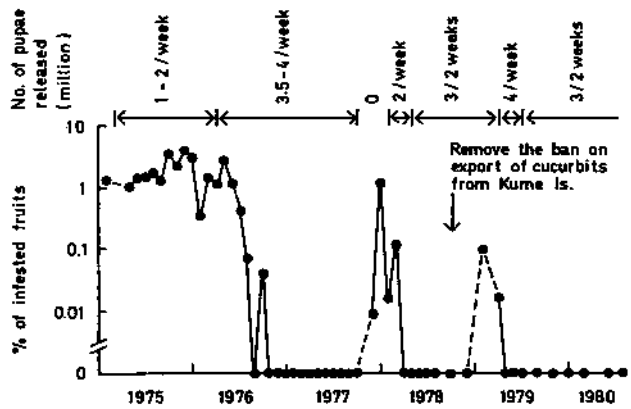

FIG. 4. Trend in percentage of wild cucurbit fruit, Bryonopsis laciniosa, *infested with the melon fly in Kume Island* [14].

OKINAWA PROJECT

Experimental eradication of the melon fly in Kume Island by SIT

The distribution of the melon fly had been limited to the islands south of the Miyako Islands up to 1970, when the fly was first recorded in Kume Island. After the invasion of Kume Island, the melon fly spread rapidly northwards. The present northern limit of distribution of the fly is the Amami Islands (Fig. 1).

In 1972, an experimental eradication project began in Kume Island [6–11]. From December 1972 through December 1974, suppressive control of the melon fly was carried out using cotton rope or fibre block saturated with cue-lure and insecticides, along with poisoned protein hydrolysate spray [6]. Since February 1975, about one million sterile pupae have been released weekly in Kume Island. However, the ratio of sterile : wild flies did not exceed one [7]. The estimation of the population density of the fly carried out before the release [12] and the calculation based on a population model [13] indicated that 4 million flies should be released weekly to achieve rapid eradication. From September 1975, the number of sterile pupae released was increased to 2 million weekly. As a result of this increase, the sterile : wild ratio became 10 : 1 in May 1976. The number of released flies increased to 3.5–4 million weekly from May 1976, after which the sterile : wild ratio exceeded 100 : 1, and the percentage of infestation of host fruits decreased to zero in August 1976 (Fig. 4). As the zero infestation level was maintained for one year, it was thought that eradication had been achieved [8]. However, when the release was stopped, re-invasion occurred at once [14]. This re-invasion was

TABLE V. DURATION OF SIT APPLICATION FOR THE MELON FLY AND THE MALE ANNIHILATION METHOD APPLICATION FOR THE ORIENTAL FRUIT FLY IN THE OKINAWA REGION

Name of islands	Area	Period	
	(km^2)	Melon fly	Oriental fruit fly
Okinawa Isls	1434.0	–	1977–
(Kume Is.[a])	(62.5)	1972–77	1977–
Miyako Isls	227.0	–	–
Yaeyama Isls	584.4	–	–

[a] Kume Island is included in the Okinawa Islands.

checked by the release of 2 million sterile flies weekly. The fortnightly release of 3 million sterile flies was maintained to prevent a re-invasion, and the ban on the export of cucurbit fruit from Kume Island was removed in September 1978. To prevent re-invasion, 3 million sterile flies were also released fortnightly in the small islands of Kerama which are located between Kume Island and Okinawa Island where the fly occurs abundantly. In Kume Island, sterile pupae were distributed with a bucket, but in the Kerama Islands they were released from a helicopter after the adult flies had emerged.

In spite of these measures, infestation of the fly was detected in January 1979. This re-invasion was checked by the weekly release of 4 million sterile flies for three months. Kume Island, which is only 80 km from Okinawa Island, has always been threatened by a re-invasion of flies from infested islands (Table V). A large project to eradicate the melon fly from all the Okinawa, Miyako and Yaeyama Islands within about 10 years is at present under way.

Oriental fruit fly eradication by the male annihilation method

The oriental fruit fly was first recorded in 1918 in Okinawa Island. The eradication project by the male annihilation method began in 1977 (Table V) [14]. Since October 1977, cotton rope (forests and fields) or rolled cotton (populated areas) saturated with poisoned methyl eugenol has been distributed by helicopter or deposited on the ground, respectively. Until the autumn of 1978, the number of flies trapped was reduced only to 1/10 of the number recorded in the same season of 1977 (Fig. 5). At this time two possible reasons for the ineffectiveness

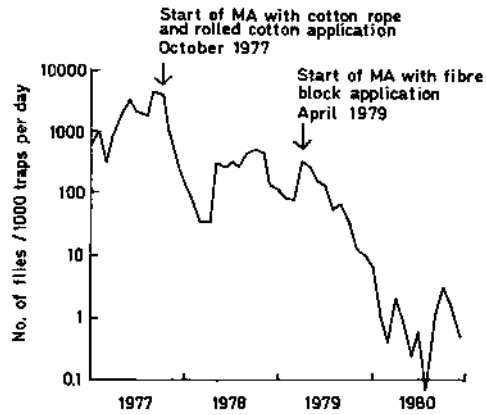

FIG.5. Trend in the oriental fruit flies caught by monitor traps in Okinawa Island after application of the male annihilation method.

of the chemicals were considered. One was the development of a resistant population such as that in the Ogasawara Islands. The other was the insufficient dose of chemicals applied. Experiments revealed that the effect of the chemicals incorporated in cotton rope or rolled cotton persisted for less than 30 days. At that time, cotton rope or rolled cotton was applied every 45 days. Experiments also indicated that the persistency of the chemicals could be extended to two to three months by using fibre block. As a result of these studies, fibre blocks have been used both for aerial and ground applications since April 1979. The dose of the chemicals applied was also increased to more than three times. As shown in Fig. 5, the number of flies trapped decreased remarkably. During 1980, the number of flies reached the level of 1/1000 compared with that before the control, and the percentage of infestation of host fruits was almost zero. This result indicated that there was no resistant population to poisoned methyl eugenol in Okinawa Island. It is expected that the eradication of the oriental fruit fly from Okinawa Island will be completed in the near future. Thereafter the next target for the project will be the Miyako and Yaeyama Islands.

TAIWAN PROJECT

Oriental fruit fly control by the male annihilation method and SIT

An integrated control project for the control of the oriental fruit fly using the sterile insect technique and the male annihilation method was started in 1975

TABLE VI. INTEGRATED CONTROL WITH THE MALE ANNIHILATION METHOD AND SIT IN TAIWAN[a]

Year	No. of districts	Area of treated orchard (ha)	No. of applications	Total no. of sterile flies released each time
1975	8	41 010	8	4 000 000
1976	12	60 590	16–20	5 000 000
1977	13	64 476	16–20	
1978	15	67 504	16–20	
1979	15	67 504	20–23	18 000 000

[a] Data from Ref. [15].

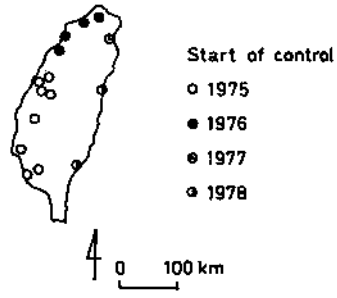

FIG. 6. Districts where integrated control with the male annihilation method and SIT was applied in Taiwan (data from Ref. [15]*).*

in Taiwan [15]. Sterile pupae were released on the ground in the citrus, mango and other host fruit plantations. The number of districts, areas and the number of flies released increased from year to year (Table VI, Fig. 6).

The sterile pupae were released three times a month in each orchard from April to the end of June and from September to November each year. From August 1979, release by aircraft was adopted.

Wicks with poisoned methyl eugenol were dropped in the orchards from the air, instead of releasing the sterile flies, when the population density of the wild flies was high.

As a result of this control the fruit losses decreased from 6.7% and 5.2% to 0.018% and 0.034% in the Ponkan (*Citrus pownensis*) and the Tankan (*C. tankanensis*) areas respectively, and from 26.3% to 2.06% in the case of mangos.

The spraying of insecticides (poisoned protein hydrolysate and Fenthion or Formothion) on the citrus and mango orchards cost NT$ 53 120 000 yearly before 1975 and only covered 36 500 ha. In comparison, expenditure for the integrated control by SIT and the male annihilation method was only NT$ 10 507 000 and covered 67 504 ha in 1979. Moreover, fruit wrapping for the control of mango had not been applied in many orchards after the integrated control, and environmental pollution from the insecticide spray ceased.

For the eradication of the oriental fruit fly, the male annihilation method was used in the Kagoshima, Ogasawara and Okinawa Projects in Japan.

In the Kagoshima and Okinawa Projects, eradication could be achieved if a sufficient dose of chemicals was used. However, re-invasion of the flies should be considered as a possibility even in the situation of islands in the sea. Thus the control area should be as wide as possible.

In the Ogasawara Project, the male annihilation method was not successful for eradication, not because of insufficient dose of chemicals but because of the possible development of populations resistant to the chemicals. Under such circumstances, the application of SIT appeared to be effective.

For the eradication of the melon fly, SIT was applied in the Okinawa Project. The estimation of the population density and the calculation of the number of released flies required for achieving the eradication should be performed before releasing the sterile flies. Such estimations were also useful in the case of the oriental fruit fly in the Ogasawara Project. In the experimental release of sterile melon flies in Kume Island, a sufficient number of flies released enabled a successful eradication to be achieved. However, re-invasion from surrounding islands occurred twice. Thus treatment should cover wide areas as in the case of the oriental fruit fly.

SIT and the male annihilation method were used for the integrated control of the oriental fruit fly on citrus and mango plantations in Taiwan. These methods reduced the infestation level of fruits. From the angle of cost/benefit, these methods were preferable to insecticide spraying, in particular with regard to pollution.

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REVIEW OF SIT RESEARCH, AND IMPLEMENTATION AGAINST PESTS OF MEDICAL, AGRICULTURAL AND VETERINARY IMPORTANCE

Session 2

EL PAPEL DE FAO/PNUMA EN PROGRAMAS DE MANEJO INTEGRADO DE PLAGAS

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Abstract–Resumen

THE ROLE OF FAO/UNEP IN INTEGRATED PEST MANAGEMENT PROGRAMMES.

The Food and Agriculture Organization (FAO) is the United Nations executing agency for technical assistance to governments and bears witness to the benefits of, and the controversies generated by, the systems used for improving the living standard of the world population. Aware of the essential importance of its activities, FAO strives to reconcile the various technologies of production of foods and fibres, safeguarding human health, the quality of the environment and the economic feasibility of production. The United Nations Environment Programme (UNEP) has joined in this noble and titanic task, the goals of which are to be achieved through the practical application of integrated pest management technologies. The work of FAO for 15 years on integrated pest management and the implementation of the FAO/UNEP Co-operative Global Programme for the Development and Application of Integrated Pest Control in Agriculture are proving to have highly promising and beneficial characteristics and great practical use. The development of integrated pest management called for the establishment of a Committee of Experts on Pesticides in Agriculture, which was reconstituted in 1966 as the FAO Panel of Experts on Integrated Pest Control with the function of advising FAO and suggesting agricultural policies to improve production with a minimum of undesirable side-effects. In 1979, the request of UNEP that the Panel of Experts should also act as its advisory body was approved, and the latter's terms of reference and name were duly modified so that it became the FAO/UNEP Panel of Experts on Integrated Pest Control. The principal tactics followed in developing and applying integrated pest management are described, including the holding of 13 technical and scientific meetings, the establishment of five working groups, the issue of 21 publications and the implementation of 23 field projects on integrated pest control.

EL PAPEL DE FAO/PNUMA EN PROGRAMAS DE MANEJO INTEGRADO DE PLAGAS.

La Organización para la Agricultura y la Alimentación (FAO) es la agencia ejecutiva de las Naciones Unidas para la asistencia técnica a los gobiernos y ha sido testigo de los beneficios y controversias de los sistemas empleados en mejorar el nivel de vida de la población mundial. La FAO, consciente de la trascendencia de sus acciones, se esfuerza en conciliar las diversas tecnologías de producción de alimentos y fibras, salvaguardando la salud humana, la calidad ambiental y la factibilidad económica de la producción. El Programa de las Naciones Unidas para el Medio Ambiente (PNUMA) se ha unido en tan noble y titánica labor, cuyas metas piensan alcanzar a través de la aplicación práctica de las tecnologías del "Manejo Integrado de Plagas" (MIP). Quince años de labor de la FAO en MIP, y la ejecución del Programa Cooperativo Global FAO/PNUMA para el Desarrollo y

Aplicación del Control Integrado de Plagas en Agricultura están demostrando ser de beneficios muy promisorios y de uso práctico muy factible. El desarrollo del MIP implicó la formación de un Comité de Expertos en Plaguicidas Agrícolas, que en 1966 fue sustituido por el Cuadro de Expertos de la FAO en Control Integrado de Plagas, con funciones de asesorar a la FAO y sugerir las políticas agrícolas hacia una mejor producción con el mínimo de efectos colaterales no deseables. En 1979 se hizo efectiva la solicitud del PNUMA para que el Cuadro de Expertos les sirviera de órgano asesor a ellos también, y se ajustaron debidamente los términos de referencia y la denominación, que pasó a ser: Cuadro de Expertos FAO/PNUMA en Control Integrado de Plagas. Se mencionan las tácticas más destacadas seguidas en el desarrollo y aplicación de MIP, lo que ha implicado la organización de 13 reuniones técnico-científicas, la formación de 5 grupos de trabajo, la edición de 21 publicaciones, a través de los 23 proyectos de campo ejecutados para aplicación de control integrado de plagas.

INTRODUCCION

La Organización para la Agricultura y la Alimentación (FAO), como agencia ejecutiva de las Naciones Unidas, da la asistencia técnica a los gobiernos en aspectos de desarrollo agrícola, forestal y de pesca, enfrentando desde su creación, en octubre de 1945, los beneficios y controversias en distinto grado de los varios sistemas empleados en su lucha por mejorar el nivel de vida en un mundo de población siempre creciente.

La FAO, consciente de esos beneficios y controversias así como de la trascendencia mundial de sus acciones, se esfuerza continuamente por conciliar las diversas tecnologías que requiere el logro de sus metas. Este esfuerzo se demuestra palpablemente en la labor conjunta que desempeña en la actualidad con el Programa de las Naciones Unidas para el Medio Ambiente (PNUMA), el cual se interesa en desarrollar los métodos de protección y producción de alimentos y fibras conservando la salud humana, la calidad del medio ambiente y la factibilidad económica de producción.

Observando que los principios y conceptos del sistema agrícola de "Manejo Integrado de Plagas" (MIP) van en completa conformidad con sus nobles objetivos, la FAO y el PNUMA han visto la conveniencia de amalgamar sus esfuerzos en apoyo al desarrollo y aplicación del MIP, que se empeña en conciliar las técnicas y medios más convenientes, facilitando vías promisorias a soluciones favorables a las preocupaciones e intereses de ambos organismos.

PRIMER INTERES DE LA FAO EN MIP (1959)

Con el desarrollo y uso indiscriminado, extensivo e intensivo de los insecticidas organo-sintéticos, a pesar de la intensificación de esfuerzos e inversiones en fitosanidad, la FAO vivió y enfrentó en el campo problemas cada

vez más frecuentes por deficiencias en control de plagas, con sus consecuencias de baja en producción en muchos casos y otra serie de efectos colaterales no deseables. Tal situación condujó a convocar en 1959 la reunión de un pequeño grupo de expertos en insecticidas, que revisó las causas, importancia y trascendencia de dichos problemas.

Basada en las recomendaciones emanadas, la FAO estimuló en su asesoría a los gobiernos la iniciación e intensificación de investigación fundamental que proveyera la forma de armonizar el uso de los insecticidas organo-sintéticos con el control biológico y prácticas ecológicas.

ESTABLECIMIENTO DEL CUADRO DE EXPERTOS DE LA FAO EN CONTROL INTEGRADO DE PLAGAS

Tres años más tarde, en 1962, ante la necesidad de una asesoría continua y especializada para el desarrollo práctico de nuevos sistemas de control de plagas más económicos y menos riesgosos, la FAO consideró conveniente la formación del Comité de Expertos en Plaguicidas Agrícolas integrado por especialistas en aspectos de residualidad, resistencia y control legal de los plaguicidas. Este Comité dió el apoyo científico y técnico necesario que aseguró el rápido progreso de la FAO en "Control Integrado de Plagas" (CIP), sentando así las bases para que en la XII Reunión de la Conferencia General de dicho organismo se le encomendara la dirección de su desarrollo y aplicación.

Cumpliendo con su labor, la FAO convocó en 1965 el primer Simposio Internacional sobre Control Integrado de Plagas, en el que 34 gobiernos representados por 85 participantes manifestaron acuerdo en el uso de este sistema, apoyando al mismo tiempo la continuidad de un grupo asesor calificado mediante el establecimiento del Cuadro de Expertos de la FAO en Control Integrado de Plagas, constituido por expertos en el uso de esta disciplina en los varios cultivos de las diversas regiones climáticas de países desarrollados o en vías de desarrollo. Este Cuadro de Expertos, establecido oficialmente en 1966 con 37 expertos de 18 países, cuenta actualmente con 55 miembros que representan a 30 naciones, de las cuales el 35% son países en vías de desarrollo.

ASOCIACION DEL PNUMA CON LA FAO PARA EL DESARROLLO DE PROGRAMAS DE CONTROL INTEGRADO DE PLAGAS

En 1974, la FAO, consciente de los avances, las bondades y la demanda de aplicación del CIP en el campo, como también de sus limitaciones en llevar a cabo ella sola esa labor titánica, y tomando en consideración la concordancia entre sus propios objetivos y los del PNUMA, con la asesoría del Cuadro de

CUADRO I. ESTADO DE AVANCE DEL PROGRAMA COOPERATIVO GLOBAL FAO/PNUMA PARA EL DESARROLLO Y APLICACION DEL CONTROL INTEGRADO EN AGRICULTURA

Cultivo	Región geográfica	Países (en proyecto)	Países (en ejecución)	Fecha inicio
Algodón	Cercano Oriente	Afganistán Grecia Irán	Pakistán Siria Turquía	Abril de 1975 Junio de 1978 en tramite
	Africa del Norte	Tanzania	Egipto Sudán	 Dic. de 1978
	América Latina	Bolivia Brasil El Salvador Guatemala México Perú	Colombia	Julio de 1981
Arroz	Asia Oriental		Bangladesh Filipinas India Indonesia Malasia Sri Lanka Tailandia	 Abril de 1975
Granos básicos	Sahel		Alto Volta Cabo Verde Chad Gambia Malí Mauritania Níger Senegal	 Enero de 1980

Expertos, propuso la concepción del Programa Cooperativo Global FAO/PNUMA para el Desarrollo y Aplicación de Control Integrado de Plagas en Agricultura; con éste se formarían expertos en la aplicación del CIP, facilitando las disponibilidades a los países en vías de desarrollo para una producción rentable de alimentos y fibras y salvaguardando la calidad ambiental.

Las continuas evidencias del progreso y necesidades del CIP respaldaron la propuesta anterior y, en 1977, el Director Ejecutivo del PNUMA, consciente

CUADRO II. REUNIONES DE LA FAO SOBRE CONTROL INTEGRADO DE PLAGAS

Fecha	Título
Oct. 1965	Simposio Internacional en Control Integrado de Plagas
Dec. 1972	Conferencia Internacional en "Ecología en relacion al Control de Plagas Agrícolas
1974	Reunión especial para la formulación del "Programa Cooperativo Global para el Desarrollo y Aplicación de Control Integrado"
Sept. 1967	Primera Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Sept. 1968	Segunda Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Sept. 1970	Tercera Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Dec. 1972	Cuarta Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Oct. 1974	Quinta Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Oct. 1975	Sexta Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Abril 1977	Septima Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Sept. 1978	Octava Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Dic. 1979	Novena Reunión del Cuadro de Expertos de la FAO en Control Integrado de Plagas
Marzo 1981	Décima Reunión del Cuadro de Expertos FAO/PNUMA en Control Integrado de Plagas

también del beneficio derivado para su organismo a través de la asesoría del Cuadro de Expertos de la FAO, solicitó que éste sirviera también como órgano asesor del PNUMA, lo que se realizó en 1979 con los consecuentes cambios de términos de referencia. A partir de entonces, el Cuadro de Expertos tomó la denominación de Cuadro de Expertos FAO/PNUMA en Control Integrado de Plagas.

En los pocos años de labor conjunta de la FAO y el PNUMA, y con el grado de ejecución del Programa Cooperativo Global (Cuadro I) aplicado a los cultivos

CUADRO III. GRUPOS DE TRABAJO EN TEMAS ESPECIFICOS

Tema	Estado
1. Uso de productos de comportamiento en manejo de plagas	Informe publicado
2. Los plaguicidas en programas de control integrado	Borrador en revisión
3. Crecimiento de la plantación y manejo de plagas	Tema demasiado amplio Modificado: Factores de crecimiento
4. Predicción y pronóstico de plagas	Abandonado. Traslape con otras publicaciones
5. La economía y el control integrado de plagas	Recomendación reciente (marzo de 1981)

de algodonero, arroz y granos básicos, los resultados han sido notablemente fructíferos y sumamente alentadores al proveer los elementos necesarios para desarrollar las estrategias agrícolas propias encaminadas hacia una producción agrícola mejorada en todo sentido, confirmando así la convicción de la practicabilidad de la aplicación del control integrado a niveles nacionales y regionales, a pesar de su complejidad.

ACCIONES IMPLICADAS EN EL DESARROLLO DE LOS PROGRAMAS DE CONTROL INTEGRADO DE PLAGAS

Los quince años de labor decidida de la FAO así como los esfuerzos conjuntos con el PNUMA para el desarrollo de control integrado a nivel mundial han implicado una serie de acciones que, cada una en su debida oportunidad, han surtido efectos decisivos en la concretización de los actuales y futuros programas de manejo integrado de plagas (MIP). Las acciones fundamentales han consistido en:

1) Discusión, clarificación y unificación de conceptos, criterios y tecnologías de control integrado a aplicar en diversas circunstancias.
2) Revisión de causas de fallos y progresos en la implantación del manejo integrado de plagas, resultando en recomendaciones generales y específicas a los varios proyectos y dando bases y orientaciones para las definiciones de políticas técnicas y crediticias en el desarrollo agrícola de las naciones.

CUADRO IV. PUBLICACIONES DE LA FAO SOBRE CONTROL INTEGRADO DE PLAGAS

Fecha de publicación	Título
1965	Proceedings of the FAO Symposium on Integrated Pest Control (3 volúmenes) Rome, Italy
1966	Informe del Simposio de la FAO en Control Integrado de Plagas (Informe N: PL/1965/15) Roma, Italia
1971	Control integrado de plagas. Qué significa – Por qué se necesita urgentemente – Qué se debe hacer – Quién lo debe hacer. FAO, Roma, Italia
1972	Proceedings of the FAO Conference on Ecology in Relation to Plant Pest Control (AGP: 1973/M/14), Rome, Italy
1972	Informe de la Conferencia de la FAO sobre Ecología en Relación con el Control de Plagas Agrícolas (Informe No. AGP/1973/m/6), Roma, Italia
1973	Manual de Control Integrado de Plagas del Algodonero (AGPP: Misc/8), Revisado en Noviembre de 1974 (Inglés, francés, español, árabe)
1974	Informe de una Reunión Ad Hoc del Cuadro de Expertos de la FAO en Control Integrado de Plagas (Informe AGP: 1974/M/8), Roma, Italia
1975	Informe de una Consulta FAO/PNUMA sobre Sistemas del Manejo de Plagas para el Control de Plagas del Algodonero (Informe No. AGP: 1976/M/3), Karachi, Pakistán
1979	Manual de Control Integrado de Plagas del Arroz, Estudio FAO: Producción y Protección Vegetal No.14 (Inglés, francés, español)
1979	Guidelines for Integrated Control of Maize Pest, FAO Plant Production and Protection, Paper No.18 (Inglés y francés – español en preparación)
1980	Introducción al Control Integrado de las Plagas del Sorgo, Estudio FAO Producción y Protección Vegetal No.19 (Inglés, francés, español)
1967	Informe de la Primera Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. PL/1967/M/7), Roma, Italia
1968	Informe de la Segunda Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1970/M/3), Roma, Italia
1970	Informe de la Tercera Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1970/M/7), Roma, Italia
1972	Informe de la Cuarta Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1973/M/5), Roma, Italia
1974	Informe de la Quinta Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1975/M/2), Roma, Italia

CUADRO IV. (cont.)

Fecha de publicación	Título
1975	Informe de la Sexta Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1976/M/1), Roma, Italia
1977	Informe de la Séptima Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1977/M/8), Roma, Italia
1978	Informe de la Octava Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1979/M/1), Roma, Italia
1979	Informe de la Novena Sesión del Cuadro de Expertos en Control Integrado de Plagas (Informe No. AGP: 1980/M/5), Roma, Italia
1981	Informe de la Décima Sesión del Cuadro de Expertos en Control Integrado de Plagas (en preparación), Roma, Italia

3) Revisión y recomendaciones sobre problemas y tópicos específicos como los de la mosca del Mediterráneo (*Ceratitis capitata*), plagas de productos almacenados, garrapatas del ganado, etc.
4) Orientación y coordinación de una investigación complementaria entre los países de una misma región, a fin de acelerar el progreso y evitar duplicaciones innecesarias.
5) Montaje y supervisión de parcelas de estudio-demostración para la aplicación del control integrado, a fin de apreciar los grados de factibilidad y beneficio, e identificar los obstáculos clave en su adopción a nivel comercial.
6) Asesoría y supervisión de la aplicación del control integrado a nivel comercial.
7) Actualización tecnológica y científica entre los diversos Países Miembros y disciplinas científicas, a través de discusiones en 13 reuniones internacionales especificadas en el Cuadro II.
8) Asignación y apoyo económico de 5 grupos de trabajo para explorar la factibilidad de temas especiales (Cuadro III).
9) Transferencia de progresos, experiencias y tecnologías desarrolladas de control integrado, contenidos en 21 publicaciones (Cuadro IV), y el desarrollo de más de 100 cursos de entrenamiento a todos los niveles.
10) Ejecución de 23 proyectos de asistencia técnica en control integrado aplicado a diversos cultivos, y distribuidos en varias partes del mundo, como se indica en el Cuadro V.

CUADRO V. PROYECTOS DE LA FAO CON APLICACION DE CONTROL INTEGRADO

Título	Iniciación	Duración (años)	País o región
Investigaciones sobre la lucha contra las plagas y enfermedades del olivo en Grecia continental, Creta y Corfú	1970	11	Grecia
Control biológico de plagas del algodón en Nicaragua	1970	3	Nicaragua
Control integrado en plagas agrícolas (granos básicos) en Nicaragua	1974	5	Nicaragua
Lucha contra el barrenillo rinoceronte del coco	1964	10	Pacífico meridional
Investigación sobre control de aves granívoras *(Quelea quelea)*	1970	6	Africa Occidental y Sudán (13 países)
Reforzamiento de los servicios de protección vegetal. Investigación y capacitación	1971	12	Tailandia
Establecimiento de un Centro de protección vegetal, Ta Chung, Taiwan	1971	3	Taiwan
Fomento de la producción algodonera	1970	3	Ghana
Instituto de Investigación Agrícola	1971	8	Etiopía
Lucha biológica contra las espumadoras de la caña de azúcar en Pernambuco y Alagoas	1968	4	Brasil
Instituto Central de Investigación de Algodón, Multan, Pakistán	1976	6	Pakistán
Control biológico de áfidos de los cereales en Chile	1977	2	Chile
Reforzamiento de la Escuela Superior Federal de Agronomía, Yaundí, Camerún	1970	5	Camerún
Reforzamiento de la capacitación agrícola de la Escuela Nacional de Agronomía, Kindia, Guinea	1970	3	Guinea
Instituto de Tecnología Agrícola de Bagdad, Irak	1980	5	Irak
Centro de Investigaciones y Capacitación para la Protección de Arroz	1962	8	Tailandia

CUADRO V. (cont.)

Título	Iniciación	Duración (años)	País o región
Control biológico del minador de la hoja del cocotero *(Promecotheca cumingi)*	1972	3	Sri-Lanka
Lucha contra el *Quelea*	1970	4	Somalia
Reforzamiento del Instituto de Capacitación Vegetal, Hyderabad	1973	5	India
Centro de Investigación y Capacitación en Protección Vegetal, Afgoi	1973	3	Somalia
Contribución de la productividad de la alfalfa	1972	7	Argentina
Producción en masa de insectos parásitos de las plagas de los cultivos en Costa Rica	1973	1	Costa Rica
Reforzamiento del Servicio de Protección Vegetal	1980	3	Burma

SITUACION ACTUAL E IMPORTANCIA DE LOS PROGRAMAS DE MANEJO INTEGRADO DE PLAGAS

Resumiendo el análisis de la actual situación científica, técnica y práctica del desarrollo, uso y aplicación del manejo integrado de plagas, derivado de los proyectos y programas en ejecución por la FAO y el PNUMA, cabe notar que:

a) En general, la investigación en control integrado tanto en los países avanzados como en los países en vías de desarrollo ha progresado suficientemente, conociéndose los elementos necesarios para el diseño de estrategias aplicables en la práctica.
b) Con las áreas de estudio-demostración de aplicabilidad del control integrado, en muchos proyectos se han afinado detalles que han resultado en la estructuración de sistemas de manejo de plagas a punto para aplicarse a nivel comercial.
c) Gracias al apoyo decidido del PNUMA con su Programa Cooperativo Global, de tipo regional, se ha podido apreciar en forma práctica la gran trascendencia y potencial de beneficios que tiene el sistema de manejo integrado de plagas en todos los aspectos: salud humana, calidad ambiental, rentabilidad agrícola, beneficios sociales, etc.

d) La suspensión de apoyo económico a muchos proyectos en el momento en que han completado su fase de investigación e inician la fase de extensión al agricultor, no ha permitido la transferencia al verdadero usuario de tecnología desarrollada, ocasionando el estancamiento, e incluso retroceso del avance logrado, e impidiendo la obtención de los verdaderos frutos a que está dirigido el sistema.
e) Las múltiples solicitudes que recibe la FAO de apoyo económico y asesoría técnica en la aplicación del control integrado a diversos niveles son atendidas sólo en una mínima parte por falta de fondos.
f) El escaso apoyo económico consentido por los organismos multilaterales ha sido un gran factor limitativo para una aplicación práctica más extensa y veloz del control integrado.

RECOMENDACIONES

Ante las anteriores circunstancias y la convicción de las bondades del uso del CIP, se someten a consideración las siguientes recomendaciones:

1) Poner de relieve los programas de CIP en sus aspectos de entrenamiento, supervisión, divulgación, etc., tanto en su forma extensiva como intensiva, así como las actividades propias y directamente relacionadas con la transferencia tecnológica al usuario mismo.
2) Expresar reconocimiento al PNUMA por la forma decidida y de gran trascendencia en que ha apoyado los programas de aplicación práctica del CIP, y darle estímulos para reforzar tan importante apoyo.
3) Asegurarse fondos para programas de larga duración.
4) Asignar lo máximo posible a elementos nacionales para la planificación y desarrollo de programas de control integrado.

BIBLIOGRAFIA

(Véase el Cuadro IV)

CONTROL OF MOSQUITOES BY THE STERILE MALE TECHNIQUE

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Abstract

CONTROL OF MOSQUITOES BY THE STERILE MALE TECHNIQUE.

Field tests on the applicability of SIT to mosquito control have been conducted since the late 1950s. Early field experiments were conducted by releasing radiation-sterilized males. Methods of chemically sterilizing mosquitoes were also developed. Genetically altered strains which are partially sterile were also developed, studied and then used in field experiments. The earliest release experiments with mosquitoes were unsuccessful in introducing sterility into natural populations or reducing insect density, but identified problems and developed methodology. A summary of the releases conducted since the 1950s is given as background and then recent tests are reviewed in more detail where population control was achieved. The advances made in understanding the dynamics of field populations of mosquitoes when subjected to SIT are also reviewed. The problems associated with SIT for mosquito control — absolute density, growth rate, migration and others —are also discussed.

The principles, theory, requirements, advantages and problems associated with the application of the Sterile Insect Technique (SIT) to insect control practices have been adequately detailed by Knipling [1,2] who conceived the unique method and demonstrated with scientific colleagues its practical application to several major economic species of insects. Since the theory behind this unique approach to control has been adequately proven, our major concern is the adaptation and development of the method to other types of insect problems. Since each insect or insect group has different characteristics in terms of habitat, density, population dynamics, behavior, biology, ecology and economic impact, the development of the method for any specific insect cannot be assured on the basis of the theory alone without considerable basic and applied research. For example, the simplest expression of the theory of SIT is:

$$F_1 = P\ (1-S)\ R$$

In this case the absolute density of any population expected to occur in any given generation (F_1) is simply the absolute density of the

population in the preceeding generation (P) whose replacement or growth rate (R) is reduced by the degree of sterility (S) induced into the parent generation by the released insects. Our prime interest is the adaption of SIT to mosquito control. Mosquitoes, as other insects, present special problems to the development of SIT, the most important of which are highlighted by the theory. There are hundreds of species of mosquitoes worldwide that are economically important because they transmit diseases to animals and people or cause severe annoyance. Most research on the development of SIT has involved a few species in the genera *Anopheles*, *Culex* and *Aedes* that are disease vectors. A requirement of SIT involves estimates of the absolute densities of populations. The amount of data on the absolute numbers of mosquitoes per unit area is very limited. Another requirement of SIT is knowledge of the growth rates of populations. Although we have considerable data on the biotic potential of some mosquitoes from laboratory studies and some data on density changes of naturally occurring populations, we have little data on the growth rates of populations reduced below carrying capacity by SIT or other control methods, and the degree of density dependent regulation and its influence on growth rate. Finally, the theory for SIT applies to populations in isolation or in areas large enough to negate the influence of immigration. Most mosquitoes can disperse over large areas while their breeding and mating sites are generally restricted to close proximity to water sources. Thus, mosquitoes present unique problems for the development of SIT. Mosquitoes generally occur at high but unknown absolute densities. They have high biotic potential which is never achieved under natural conditions and they can migrate over considerable distance.

The purpose of this paper is to review the field tests conducted on SIT with mosquitoes and indicate the advancement and practical problems associated with these experiments as well as the status of the development of SIT.

Research toward the development of SIT for mosquito control has been conducted with limited resources over the past 25 years. Methods of sterilizing both males and females of several species by exposure to radiation or chemicals were developed. Genetic mechanisms which involved partial sterility and therefore could, theoretically in some types, be transmitted to natural populations were described and propagated for laboratory experiments. These genetic mechanisms offered the potential of population reduction because of partial sterility or population replacement if sufficient insects could be released.

These developments led to field experiments reported in the literature from 1962 to date testing the potential and examining the problems associated with SIT with about 10 species of mosquitoes. Tables I and II are included to indicate the extent of these field experiments and supply a list of references to those interested in the area of development. This paper will review the earlier or preliminary experiments with only limited comments, and then concentrate on later field experiments where population control was achieved.

TABLE I. SUMMARY OF FIELD EXPERIMENTS ON SIT AGAINST VARIOUS SPECIES OF MOSQUITOES

Year	Location	Species	Sterilizing agent	Reference
1962	Florida, USA	*Anopheles quadrimaculatus*	gamma	3, 4
1962	Florida, USA	*Aedes aegypti*	gamma	6
1962	New Delhi, India	*Culex p. quinquefasciatus*	gamma	7
1970	Florida, USA	*Culex p. quinquefasciatus*	thiotepa	8, 9
1974	El Salvador, CA	*Anopheles albimanus*	bisazir	10, 11, 12, 13
1975	New Delhi, India	*Culex p. fatigans*	gamma	14
1976	New Delhi, India	*Aedes aegypti*	thiotepa	15
1976	New Delhi, India	*Culex p. quinquefasciatus*	thiotepa	16
1977	Florida, USA	*Culex p. quinquefasciatus*	gamma	17
1978-9	California, USA	*Aedes sierrensis*	gamma	18
1980	California, USA	*Culex tarsalis*	gamma	19
1980	El Salvador, CA	*Anopheles albimanus*	bisazir	20

TABLE II. SUMMARY OF FIELD EXPERIMENTS ON SIT WITH RELEASE OF GENETICALLY ALTERED MOSQUITOES AGAINST VARIOUS SPECIES OF MOSQUITOES

Year	Location	Species	Mechanism	Reference
1967	Rangoon, Burma	*Culex p. quinquefasciatus*	cytoplasmic incompatibility	21
1970	Bobo Dioulasso Upper Volta	*Anopheles gambiae* complex	sterile hybrids	22
1972	Montpellier, France	*Culex pipiens pipiens*	translocation	23
1973	New Delhi, India	*Aedes aegypti*	translocation complex	15
1975&6	Florida, USA	*Aedes aegypti*	translocation	24, 25
1976&7	Kenya, Africa	*Aedes aegypti*	translocation	5
1980	Lahore, Pakistan	*Culex tritaeniorhynchus*	translocation	26
1980	California, USA	*Culex tarsalis*	translocation	27, 28

The first field experiments on SIT using irradiated male mosquitoes were conducted in Florida, USA,and New Delhi, India. Neither led to the demonstration of sterility in wild populations or population reduction, but each contributed information to the problem. The lack of positive results after releasing *An. quadrimaculatus* males in Florida [3] led to research studying the mating behavior of colonized insects under field conditions. This research [4] developed techniques for such studies and demonstrated assortative mating between the colonized strain and the wild strain precluding the use of the laboratory strain for SIT. Since that time similar problems with colonized material used in release experiments have been reported with *An. culicifacies* and *Cx. tritaeniorhynchus* in Pakistan and *Cx. tarsalis* in California [5].

In at least two of these cases it appears that the problems were associated with rapid selection during colonization. Although this has not happened with a majority of mosquito species which have been studied, it is an important consideration for other species. In the releases of *Ae. aegypti* in Florida [6] mechanical methods of separating females from males in the pupal stage prior to release were demonstrated, a technique which has been used successfully with both *Culex* and *Aedes*. Preliminary studies in India [7] with *Cx. p. quinquefasciatus* were discontinued because of reaction of people who thought releases were causing mosquito problems even though only sterile, non-biting males were being released.

The first successful mosquito release experiments reported were in Florida against *Cx. p. quinquefasciatus* [8,9] and in El Salvador against *An. albimanus* [10,11,12,13]. These experiments were considered successful because they induced significant levels of sterility into natural populations. Population reduction occurred and sterility increased as population density was decreased. It was also possible to estimate absolute densities of mosquitoes, the dynamics of the populations, the effects of density-dependent regulation in response to control by SIT and to fit the results to SIT theory [8,9,13]. However, these release experiments were conducted in small isolated areas with relatively low density populations. They were not subjected to the problems of immigration.

A large project in India from 1969-1975 conducted cooperatively by the World Health Organization and the India Council of Medical Research with Public Law 480 support funds through the U.S. Department of Health, Education and Welfare studied the release of radiated and chemosterilized males of *Cx. p. quinquefasciatus* and *Ae. aegypti* [14,15,16]. The experiments developed methods of mass production, sterilization, handling, mechanical separation of sexes, testing and release. Essentially they were able to demonstrate the production and release of competitive males and the establishment of sterility in wild populations. Unfortunately, the occurrence of immigration clouded the effect on population density.

In Florida the feasibility of controlling an isolated, island population of *Cx. p. quinquefasciatus* by the release of males sterilized by 10 kR of gamma radiation was demonstrated [17] by daily releases over 13 weeks even though the released males were estimated to be only one-fourth to one-half competitive.

In California [18] field studies in large tents were conducted with *Ae. sierrensis* to study mating competitiveness of males sterilized by gamma radiation with results sufficiently promising to consider proceeding to field trials. Another study with *Cx. tarsalis* males sterilized by gamma radiation indicated some impact on the fertility of the field population [19].

Finally, an extensive study on the release of sterile *An. albimanus* males in a 150 km^2 area in El Salvador [20] was undertaken from 1975-1978. This study will be considered in more detail later in the paper.

Table II lists field experiments with genetically-altered mosquitoes that have been conducted since 1967. The experiment in Rangoon, Burma [21], in which cytoplasmically incompatible males were released, claimed and has been cited as an example of the eradication of *Cx. p. quinquefasciatus* by releases. However, the ratio of released to wild insects never exceeded 1:1 during the releases. Normally according to the theory of SIT one would expect the ratios to increase if a constant number were released. In this case the number released must have been reduced to match the decreasing population. How this was accomplished was not discussed in publication. At any rate it is difficult to understand how a population of *Cx. p. quinquefasciatus* could be reduced to elimination with no evidence of the occurrence of density dependent regulation which would be reflected in increasing population growth rates to levels greater than 1X per generation. In other words this population had a potential growth rate no greater than that required to replenish existing numbers even at reduced densities. The other experiments deal with the release of sterile hybrids or translocation complexes and illustrate that such insects are relatively competitive under field conditions or can introduce the genetic material into field populations.

The remainder of this paper will deal in more detail with release experiments against two species of mosquitoes, *Cx. p. quinquefasciatus* and *An. albimanus*, where releases were made of chemically sterilized males into native populations. Choosing these two species and the particular experiments involved resulted primarily because our laboratory was involved and we are more familiar with design and results. Additionally, population reduction to near elimination occurred in two field experiments with *Cx. p. quinquefasciatus* and *An. albimanus*, and a larger scale trial against *An. albimanus* highlighted major problems in larger areas.

Patterson et al. [8] reported the results of a ten-week release experiment in which *Cx. p. quinquefasciatus* males sterilized by exposure as pupae to thiotepa were released into a native population on a small island. Table III is a reproduction of their published results. There is no doubt about the effect of the males since the sterility increased from 0 to 95% as the population, as measured by egg raft reduction, decreased by 96% and reached the point where the source of the few fertile eggs could not be differentiated between a few migrant fertile females or indigenous females. During the final four weeks of this experiment egg rafts were completely sterile on 19 of 28 days. These authors used a unique and simplified method of estimating the density of the wild population. A mark (females)-release-recapture (egg rafts) experiment was run immediately after the release experiment to determine the fraction of egg rafts laid in their sampling ovitraps compared to natural sites. Then, they reasoned that, with a sex ratio of 1:1, the number of females (Nx) or males emerging onto the island each day could be calculated from

TABLE III. RELEASE, STERILITY AND CONTROL DATA FOR THE STERILE MALE RELEASE EXPERIMENT WITH *Cx. p. quinquefasciatus* ON SEAHORSE KEY

The ovitraps collected about 50 percent of the total number of egg rafts that were oviposited by females each day; these egg rafts were destroyed (from Patterson et al. [8]*).*

Release data			Egg raft data				Reduction in No. of egg rafts collected (%)	
Generation	Sterile males released (No./day)	Sterile: normal males	Laid in ovitraps (No./day)	Expected to be sterile (%)	Actually sterile (%)	Sterile or destroyed (%)	Total	Fertile
1	0		228		0	50		
2	8 400	3:1	146	75	62	81	36	75
3	13 000	4:1	151	80	85	92	34	90
4	11 000	12:1	47	92	82	91	79	96
5	18 000	100:1	9	99	84	92	96	99.4
6	16 000	100:1	8	99	95	97.5	96	99.8

TABLE IV. NUMBERS OF *An. albimanus* FEMALES COLLECTED AT NIGHT AT ESTABLO APASTEPEQUE IN 1968, 1971 AND 1972
(after Lofgren et al. [12]*)*

Month	Average number females/collection in 1968	1971	1972
Jan	197	NC*	161†
Feb	10	9‡	109
Mar	1	7‡	10
Apr	0	NC	21
May	10	26	34
Jun	181	42	22
Jul	114	67	22
Aug	170	122	7
Sep	1 062	593	0
Oct	1 403	1 311	2
Nov	912	403	24
Dec	68	181	15

*NC, no collection.
†January 1973 collection was 191.
‡Collection made in the morning.

the number laying egg rafts (Ny) through an estimate of the preoviposition time (D) and the average daily survival (S):

$$\frac{Ny}{Nx} = S^D = 0.75^8 = 0.10$$

Using such a method they calculated the ratio of sterile to fertile males during releases and compared the expected to actual sterility essentially demonstrating the fit of the field data to the theory for SIT. Weidhaas et al. [9] used this data to calculate the growth rates of the population per generation while its density was being reduced by the release of sterile males. In two successive years the population growth rates were in the order of 1X just prior to the release and increased to 10X when the population was greatly reduced. The results were as follows:

Year	Generation growth rate 0	1	2	3	4
1968	1.4	1.0	5.0	10	10
1969	1.3	5.0	5.0	2.5	10

That density regulation was an important consideration is evident from the increase in growth rates with population reduction. The maximum value observed (10X) is much below the biotic potential

of this mosquito. Referring to Table III one can see that it was possible to obtain population suppression with a starting ratio of three steriles to one fertile male even though the eventual growth rate reached 10X.

A similar test in a larger area (15 km^2) against a malaria vector, *An. albimanus*, was conducted in an essentially isolated area in El Salvador [10,11,12,13]. Males were sterilized with bisazir, *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide, and released in numbers of about 14 000 per day during the first two weeks and then 30 000-40 000 during the remainder of the five months of releases. Table IV is a reproduction of the results [12] indicating the effect of the releases on female density. The population was reduced to an undetectable level during September. Interestingly, Lofgren et al. [12] reported that the levels of *An. pseudopunctipennis* larvae followed a normal seasonal cycle in the same area during the test period. Percent sterility increased rapidly, plateaued at 50 to 70%, then reached 100%. Weidhaas et al. [13] made estimates of the absolute densities of the native females and the growth rates of the population during the release period. Over one-month periods (used to approximate a generation time) the results were as follows:

	Monthly period				
	1	2	3	4	5
Growth rate	0.3	1.4	1.5	1.3	-
No. of females	14 400	2 400	1 200	300	75

There is little evidence that this population exhibited high levels of density-dependent regulation in that the growth rates did not increase to high levels as the population was reduced. These authors used the data to make estimates of survival of immature and adult stages, to explain the lack of significant density regulation and to construct models of population dynamics and vector capacity.

A much more extensive study in El Salvador followed this original test and was conducted from 1975 to 1979 [20]. The details of the test are given in this paper and others cited by the authors. The purpose here is to highlight the problems, solutions and results reported during this large test. The original experiment area consisted of about 150^2 km with an indigenous population of *An. albimanus* and active malaria transmission. A warehouse was leased to mass-produce this mosquito and production was started from material colonized from the test area. Innovations in rearing such as thermostatically controlled heat tapes under larval rearing trays and volumetric measurements of dried eggs for controlling the number of larvae per tray [29] along with controlled diets made possible the production of 1 000 000 mosquitoes per day (half males and half females). However, the separation of females from the males for release created serious problems. Mechanical methods of separation in the pupal stage left 15% females in the release material. Holding emerged adults

in cages and killing the females by placing citrated blood containing malathion in membranes in the holding cages improved the situation and a dual system - mechanical plus feeding - was adopted. The lack of any positive results when releases were made in the large area was shown to be a result of loss of vigor and competitiveness caused by excessive handling prior to release and age of the released insects. A genetically-engineered strain (MACHO) had been developed and tested in Gainesville [30] and was sent to El Salvador. This strain was constructed so that when eggs were treated with propoxur, susceptible females were killed while resistant males survived for rearing and release. The rearing system in the factory was adapted to producing this strain. In field tests the strain was demonstrated to be competitive (ca. 80%) [31] and the production of males was increased to over 1 000 000 released per day; an increase of 4- to 5-fold over previous efforts without the MACHO strain. Dame et al. [20] reported that the release of these males in larger numbers improved results but did not provide complete control. Consequently, they reduced the size of the test area to ca. 20^2 km and created a barrier zone. They reported that population growth in the treatment area was held to 1.7-fold in December and 0.6-fold in January compared to increases of 7-fold and 21-fold in a check area. They equated these levels to a 76% and 97% control. In February and March they combined weekly larvicidal treatments with methoprene with the release of sterile males and obtained further population reduction. Complete control was not obtained because of the influx of migrants which was confirmed by release and recapture experiments.

Thus we can conclude on an optimistic but cautious appraisal of the development and future of SIT for mosquito control. Field experiments have demonstrated that the method can reduce mosquito populations in conformance with SIT theory. The experiments conducted to date have been an invaluable aid in developing practical and useful information on the population dynamics of some species and the dynamics of populations when subjected to management procedures. Such information has allowed the construction of models relating population control and dynamics. In situations where densities are reduced or immigration can be managed the method should be a helpful addition for use in management schemes that combine a variety of control technologies directed at population control or management.

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THE STERILE INSECT TECHNIQUE IN THE CONTROL OF THE SCREWWORM

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Abstract

THE STERILE INSECT TECHNIQUE IN THE CONTROL OF THE SCREWWORM.

Application of the sterile insect technique (SIT) has been successful in the eradication of the primary screwworm, *Cochliomyia hominivorax* (Coquerel), from Curaçao, the south-eastern United States of America and Puerto Rico. Although the eradication programme in the southwestern United States suffered serious setbacks in 1972–76, a current joint effort of the United States and Mexico has suppressed populations of screwworms north of the 24th parallel to the extent that only seven cases have been reported in the United States since November 1979. This recent success has been attributed to (1) an improved quality of the sterile flies being released because production strains are being changed more frequently, and methods used to transport irradiated pupae from production centres to distribution centres have been improved; (2) the use of the chemical attractant, Swormlure, for surveillance and for production of a toxicant-bait system for population suppression; (3) an increase in the numbers of sterile flies being produced at a rearing facility in southern Mexico, thereby allowing a larger area to be covered with sterile fly releases; (4) better distribution of the sterile flies through the use of small aircraft and narrow swath widths; and (5) improved co-operation from livestock producers in preventing untreated wounds. The present goal of the joint programme is to establish and maintain a sterile fly barrier across the isthmus of Tehuantepec in Mexico by 1983.

The primary screwworm, *Cochliomyia hominivorax* (Coquerel), is an obligate parasite of the flesh of living warm-blooded animals present in the Americas. The gravid female oviposits 200–300 eggs near an open wound on the host animal. After hatching within 8–12 h, the larvae begin feeding on the living flesh and continue development for 4–9 days before dropping from the host to pupate in the soil. The duration of the pupal and adult stages is dependent upon environmental conditions but a generation cycle is approximately three weeks.

The screwworm does not diapause and usually does not survive the winter in the United States of America. However, at times screwworm infestations have ranged as far north as South Dakota and Illinois [1]. Untreated infestations, especially infestations from multiple ovipositions, may result in the death of the host.

The economic impact of screwworms on the livestock industry in the United States has been estimated to be as great as 100 million dollars annually [2] and at least 30 million dollars in Mexico (Personal communication, K. Ellis, Information Office, Joint U.S.-Mexico Screwworm Eradication Program). These estimates include expenses incurred in surveillance and treatment as well as losses from deaths or in market value. Benefits to wildlife populations have not been estimated.

The purpose of this presentation is to review the application of the sterile insect technique (SIT) to control the screwworm. Also, some of the more recent contributions of the supporting research programmes will be discussed.

HISTORICAL AND CURRENT STATUS

Status reports of the success or the lack of success in the efforts to eradicate the screwworm through the use of the SIT have been published [1, 3–5]. These reports discussed the successful eradication of this pest of man and livestock from the island of Curaçao, N.A., in 1954 and from the southeastern United States in 1959. Since that time, the island of Puerto Rico (1975) and Baja California (1979) have been declared to be free of screwworms. In addition, Curaçao was again eradicated after a reinfestation had occurred.

An eradication programme was initiated in the southwestern United States in 1962 with the goal of establishing a barrier along the 1500-mile border with Mexico. The programme was successful in minimizing the effects of screwworms flies invading from Mexico until 1972 when over 95 000 infestations were reported (Table I). The numbers of cases reported in the following years were smaller but not to the extent experienced before 1972. Possible causes were advanced for the lack of success, including (1) environmental conditions, modified animal husbandry practices, (3) loss of competitiveness of the sterile males either from mishandling or from genetic deterioration, (4) genetic changes in native populations, (5) improper distribution of sterile flies and (6) insufficient numbers of sterile flies [6]. The soundness of the sterile male principle was challenged [7], and the need for the genetic monitoring of native and laboratory populations of screwworms was emphasized [8, 9].

Those challenges that could be affected by man were taken up by the USDA and its co-operators. In addition, during 1976 a sterile fly production facility constructed in southern Mexico became an operational part of a joint United States-Mexico screwworm eradication programme. As a result, the numbers of cases in the United States have been drastically reduced since 1977, with the exception of 1978 when there was an outbreak in Arizona. Since November 1979, only seven cases have been reported in the United States. Also, areas in Mexico north of the 24th parallel are practically free from screwworm. In previous years these areas have provided a source for reinfestation of the United States.

TABLE I. LABORATORY-CONFIRMED SCREWWORM CASES IN THE UNITED STATES OF AMERICA, 1962–81

Year	Cases
1962	50850
1963	7168
1964	400
1965	1062
1966	1898
1967	872
1968	9877
1969	219
1970	153
1971	473
1972	95642
1973	14976
1974	7267
1975	16370
1976	29671
1977	468
1978	7230
1979	90
1980	2
1981 (to June 15)	5

The recent success experienced by the eradication effort has been attributed to several factors: (1) an improved quality of the sterile flies being released because production strains are being changed more frequently, and methods used to transport irradiated pupae from production centres to distribution centres have been improved; (2) the use of the chemical attractant Swormlure for surveillance and for the production of a toxicant-bait system for population suppression; (3) an increase in the numbers of sterile flies being produced at the rearing facility in southern Mexico, thereby allowing a larger area to be covered with sterile fly releases; (4) better distribution of the sterile flies through the use of small aircraft and narrow swath widths; and (5) improved co-operation from livestock producers in preventing untreated wounds.

Current operations of the eradication have been primarily conducted in Mexico since the phase-out of the production facility in the United States in January 1981. The ecological diversity of Mexico presents a challenge to the continued success of the eradication programme and research is an integral part of the programme.

RESEARCH FOR THE ERADICATION PROGRAMME

The development of an attractant for screwworm adults from chemicals associated with bacterial decomposition of proteins or amino acids provided a tool to improve the effectiveness of research and eradication programmes [10]. The attractant, named Swormlure, was more specific for primary screwworm flies and more attractive to male screwworms than was the standard bait of decaying liver. The use of Swormlure-baited traps became an efficient method to survey and monitor populations of native and released screwworms [11].

The subsequent development of a toxicant-bait system which incorporated Swormlure provided a system that is highly complementary to the SIT [12]. The system (called SWASS for ScrewWorm Adult Suppression System) is more efficient when target population levels are high whereas the SIT is more effective when target population levels are low. The complementary nature of these two systems was demonstrated by the swift eradication of a screwworm population that had reinfested Curaçao [12]. The adult population was suppressed 65–85% after aerial treatments of SWASS for 15 weeks. Thereafter, the distribution of sterile flies effectively eradicated the population after 10 weeks of releases.

The use of SWASS has become an operational part of the control programme.

Bush and Neck [9] reported that dramatic changes in the frequencies of certain electrophoretic variants occurred after screwworm strains were introduced into mass production. Their data suggested that rearing conditions may have exerted a selection pressure to cause these changes. Subsequent studies have indicated that the introduction of recently colonized strains into mass production probably had not been accomplished without contamination from the gene pool of the outgoing strain (Whitten, unpublished data), thereby creating additional potential for selection towards laboratory adaptation. Therefore, recent strain introductions have been made with an increased emphasis on quality, and subsequent allozyme monitoring has indicated that genetic changes occurring during production were less dramatic and that insect quality has improved [13].

The current policy concerning the timeliness of strain changes for mass production is to introduce a recently colonized strain on an annual basis. This policy was implemented primarily to reduce the consequence of genetic deterioration that could result in the loss of competitiveness among the sterile males.

Although the recent success of the programme gives little indication of the presence of mating incompatibilities between sterile males and native females in the field, research priorities have been placed on investigations to detect such occurrences. These investigations include studies of screwworms from different geographical regions of Mexico and Central America with regard to classical taxonomy (external morphology), population structures (chromosome morphology, isozyme analyses, host preferences and laboratory mating studies) and field evaluations of candidate strains to test for mating effectiveness.

In general, the genetical analyses have indicated that considerable variation exists among individuals within as well as between local populations. This variation was expressed primarily as polymorphisms in chromosome structures and among enzymes of certain enzyme systems. A deficiency of chromosomal heteromorphs among individuals sampled from a population in northeastern Mexico was suggestive of the presence of non-random or assortative mating [14]. However, the population was eradicated during 1979.

Laboratory mating tests and field tests in which sterile flies of strains derived from certain geographical regions were released in a distinctively different region did not reveal any presence of mating incompatibilities. In the field tests, the rate of sterility among egg masses collected within the area of sterile fly releases exceeded 70% during the fourth week of releases.

At present a field test is being conducted in southern Mexico to evaluate the mating efficiencies of males from a narrow genetic base (single egg mass) strain and those from a broader genetic base (multiple egg mass) strain.

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STERILE INSECT TECHNIQUE IN CODLING MOTH CONTROL

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Abstract

STERILE INSECT TECHNIQUE IN CODLING MOTH CONTROL.

Exposure of mature pupae or adult codling moths, *Cydia pomonella* (L.), to 30—40 krad of gamma radiation induces a high level of sterility in the male and complete sterility in the female without seriously affecting behaviour except for sperm competitiveness which is drastically reduced. Substerilizing doses (below about 25 krad) have very little adverse effect and induce a higher level of sterility in the F_1 male than in the irradiated male parent. The most satisfactory method of measuring the population density of native moths is by examining fruit for larval exit holes. Population increase per generation depends largely on evening temperatures during the moth's reproductive period. The codling moth is a sedentary species, and its distribution is very uneven in commercial orchards. Neglected host trees must be sprayed or destroyed to avoid reinfestation of sterile insect release orchards with immigrant moths. Laboratory-reared moths may be marked externally with fluorescent powders or internally with calco oil red without adverse effects. Mass rearing is still unreliable and expensive, and prolonged colonization affects the insects' behaviour. Successful codling moth suppression was achieved in North America and/or Europe by release of sterile males, sterile females or sterile mixed sexes; by substerile males; and by F_1 male progeny (released as diapausing larvae) of substerile males X untreated females. Arthropod predators and parasites held aphids and spider mites at noninjurious levels in most insect release orchards, but leafrollers eventually built up to damaging numbers. The sterile insect technique for commercial control of the codling moth is not feasible at this time because of high costs.

The codling moth, Cydia pomonella (L.), is one of our most destructive apple pests. At present, it is controlled effectively by persistent chemical sprays but because of the problems associated with the use of such compounds a biological control method, such as the sterile insect technique (SIT), is urgently needed.

Studies on the SIT for control of this insect were started in Canada in 1956, in the U.S.A. in 1959, and shortly thereafter in many European countries. The research findings are reviewed in this paper.

1. PRE-STERILE MOTH RELEASE CONSIDERATIONS

1.1. Sterilization

The codling moth has been sterilized by heat, chemicals and ionizing radiation. Heat-treatment does not appear promising;

sterilizing exposures were too injurious [1,2]. Chemosterilization of the adult male moth with tepa [3], thiotepa or tretamine [4] was effective but it seems unlikely that Government regulatory agencies will sanction the use of such chemicals in fruit protection because of their carcinogenic, mutagenic and/or teratogenic properties.

Codling moths are usually sterilized by gamma or X-rays. As in other species, the radiosensitivity of this insect is affected by several things. Immature stages are more easily damaged than the mature pupa or adult [5]. Anoxia has a moderating influence; the sterilizing effect of 25 krad administered in air was about equal to that of 40 krad in CO_2 [6] or N_2 [7].[1] A daily rhythm in radiosensitivity has been observed; exposure of adult moths to 40 kR of X-radiation at the time when they were most active (9 p.m. to 1 a.m.) markedly decreased the insects' longevity compared with exposures at other times of the day [8]. Dose rate is also important; exposure of male pupae to 30 krad at dose rates of 44 and 308 rad/sec induced 87 and 99% sterility respectively [9]. Evidently, radiosensitivity may vary between codling moth colonies; White et al. [10] found that irradiated (30 krad) apple-reared males mated much less often than irradiated males reared on artificial diet.

Because radiosensitivity is affected by several factors, it is often difficult to compare radiation results from one laboratory to another. Nevertheless, a reasonable generalization is that exposure of the adult moth or mature pupa to 30-40 krad induces (on the basis of egg hatch) 90-99% male and 100% female sterility, but the initial level of male sterility decreases with time and number of matings [7,11,12,13]. At this dose range, the fecundity of the irradiated female is drastically reduced [5]. Adult longevity [5,6,14] and the normal daily rhythm of mating activity [10,15] are usually unaffected. Mating propensity is increased in the female and decreased in the male [16,17] but this radio-induced change frequently goes unnoticed because of faulty experimental design [6]. A mating with a sterile male does not always elicit a normal ovipositional response [11] and it does not satisfy the female's mating propensity as effectively as a fertile mating [18].

The effect of high doses of irradiation on the transfer of sperm and accessory gland fluid (which may trigger oviposition) is not clear. Even with untreated males, spermatophore transfer does not guarantee transfer of eupyrene sperm or oviposition, but these malfunctions occur significantly more often with sterile (30 krad) males [11,12]. When sperm transfer does occur, the amount of eupyrene sperm that reaches the spermatheca seems to be about the

[1] 1 rad = 1.00×10^{-2} Gy.

same for sterile and fertile males but the sterile sperm takes longer to get there [19].

Most [e.g. 20,21] though not all [22,23] reports indicate that fully sterilizing doses of irradiation reduce the flight and olfactory response of the male whereas somewhat lower doses (ca. 30 krad) do not [24].

The competitiveness of sterile codling moth sperm is very poor. With nonirradiated moths the last mating is the effective one. However, if a female mates with a sterile (40 krad) male after she has already mated with a fertile one, she continues to lay mostly viable eggs [25,26]. Suski [27] found that at a somewhat lower dose (30 krad) sperm competitiveness was 0.4 of that of nonirradiated sperm. Since sterile sperms compete very poorly, population suppression by sterile male codling moths is largely dependent on the proportion of 1st matings with the sterile males and on the ability of such matings to trigger oviposition.

Because the overall competitiveness of sterile codling moths was found to be disappointingly low in laboratory and field cages [25,26,28] and even lower in the field (in British Columbia a ratio of ca. 20 sterile:1 wild male is required to prevent population increase [29]), many laboratories started to investigate the effects of substerilizing doses of irradiation. Unlike sterile sperm (40 krad), those from 25 krad males (ca. 85% sterile) were found to be almost as competitive as nonirradiated sperm in egg fertilization [18]. Also, matings with 25 krad males induced normal oviposition and satisfied the mating propensity of native females. Competitive tests in which the irradiated moths were added to cages with nonirradiated moths showed that 25 krad moths (85% male and 100% female sterility on the basis of egg hatch) were far superior to the 40 krad (98% male and 100% female sterility) insects [30].

The effects of much lower doses of irradiation were investigated with the view of utilizing the phenomenon of inherited sterility. (Earlier work [26] had shown that the F_1 adults of 30 krad males x nonirradiated females were mostly male and they were more sterile than the irradiated male parents.) The Swiss at Changin concentrated on the effects of a 10 krad dose [21,31]. When male moths were irradiated at this dose and caged with untreated females, mating, egg laying and adult longevity were unaffected, and egg hatch was ca. 55% (vs. 82% with nonirradiated insects). About 184 evidently healthy male and 42 female moths were produced from 1000 eggs. Olfactory response and mating propensity of the F_1 males were significantly reduced but longevity was unaffected. Matings of F_1 males with normal fertile females induced egg laying about as effectively as in the untreated controls. Sterility in the F_1 males (based on egg and 1st instar

mortalities) was greater than in the F_1 females (95 and 90%, respectively). Data on inherited codling moth sterility from other laboratories [e.g. 6,32,33] vary in detail but are essentially similar to those from Changin.

1.2. Population density and eclosion

Absolute population densities of overwintered codling moths are usually estimated by examining a known proportion of the fruit crop for exit holes of mature larvae. Each hole represents a diapausing larva potentially capable of overwintering. A correction is made for overwintering mortality which is influenced mainly by: availability of cocooning sites [34], abundance of insectivorous birds [35] and insect parasites and predators [36,37], disease incidence [38], winter temperatures [39] and snow cover [40]. Since these factors vary considerably between fruit-growing areas and between years, detailed mortality data are needed for each SIT program. An approximate measure of absolute moth density throughout summer is possible by mark-release-recapture methods, but their accuracy is subject to many errors. Pristavko [41] found that under his conditions the error was less than 10% but I think such accuracy is unusual. The relative population is sometimes measured by use of corrugated paper bands to capture cocooning larvae [42,43].

The start and rate of eclosion must be forecast so that laboratory rearing can be programmed well in advance to provide the numbers of sterile insects required to maintain the projected ratio of sterile to wild moths. The degree-day method of measuring these parameters is accurate [44] but hardly provides enough lead time to avoid over or under production of sterile moths. However, after accumulating eclosion data for several years, investigators should be able to predict the start and rate of eclosion fairly accurately. In British Columbia eclosion starts in late April or early May (pink-bud stage of 'McIntosh' apple) and usually peaks 3-4 weeks later [30]. Since up to ca. 50% of the overwintered insects may eclose in a 1-week period, provision must be made for this when planning rearing and release strategies.

1.3. Population increase per generation

This is one of the most important, variable and difficult to predict of the many factors affecting codling moth control by the SIT. In British Columbia the increase in numbers of this pest during the 1st generation is seldom greater than 3 or 4 fold. This modest increase is due to unfavorable weather (low temperatures, relatively high rainfall and high winds) during the mating, ovipositional and egg laying periods [45]. There is about

a 10-fold increase in the 2nd generation, though this can be appreciably greater if there is a prolonged period of hot weather [46]. Rate of increase per generation may be appreciably different in other fruit-growing areas but it will be largely dependent on evening temperatures during the moths' reproductive period.

It is possible that the usual rate of population increase is accelerated when man-made methods of control, like the SIT, reduce populations to very low levels. Certainly one would expect regulatory factors such as bird and insect predation to be less efficient at low codling moth densities. However, sex ratio changes in favor of the female, which would accelerate population increase and make control by the SIT more difficult, have not been observed in British Columbia [18]. More research is needed to determine whether the inability to suppress codling moth populations below a certain level by the SIT [47] is due solely to immigration or whether it is also due to increase of reproductive rate at low population densities.

1.4. Dispersal and distribution

In order to plan effective release strategies, both the dispersal rate of the sterile moth and the distribution of the wild population must be known. Most male codling moths disperse only ca. 50–60 m during their life span [e.g. 41]. However, some individuals fly much farther. In British Columbia, 0.8% of 3000 released males were recovered 3 km from the release site [30] and in Switzerland small numbers dispersed up to 8 km [48]. Much less is known about female dispersal because of lack of a good lure. However, release of fertile moths in orchards and subsequent fruit examination for F_1 progeny, has shown that the female is much more sedentary than the male. In one British Columbia experiment more than 90% of the infested fruit was within a radius of ca. 25 m from the point of moth release [46].

The distribution of wild codling moths is most uneven in commercial orchards. Hot spots of infestation can be established sometimes by use of sex pheromone traps [49], but fruit examination for larval damage is usually required when the population is at low ebb [46].

1.5. Pre-release suppression of the wild population

I cannot overemphasize the importance of reducing the wild population to low levels before the start of sterile moth release. If the population is not at a manageable level it should be reduced by chemical sprays. Also, since a large apple tree is capable of producing several thousand codling moths, a concerted

effort must be made to locate and spray (or preferably destroy) every neglected host tree.

1.6. Marking and releasing the sterile insects

The released sterile moths should be marked to provide information on dispersal, survival and ratios of sterile:wild males. External marking with fluorescent powders [50] has proved reasonably satisfactory but care must be taken to avoid overdusting which reduces the male's olfactory response. Occasionally, the marking powder contaminates trapped wild insects with consequent misidentification. Internal marking by adding calco oil red to the larval diet [51] eliminates the extra handling procedure required with external marking, is evidently harmless if the dye concentration is not unnecessarily high, and avoids any possible chance of misidentification.

Release of sterilized moths from ground stations, preferably those that protect the insects from rain, birds and ants [30], is adequate for small areas but release from wheeled vehicles or helicopter is more appropriate for large programs [52]. Release from fixed-wing aircraft has been less successful [53].

Because of the sedentary nature of the codling moth, the method of release must in itself ensure adequate distribution of the sterile insects. Twelve release stations/ha and flight paths ca. 30 m apart have proved adequate [30].

1.7. Rearing and insect quality

A few laboratories have developed artificial diets and procedures that are reasonably suitable for mass rearing the codling moth [e.g. 51,54] but costs are high and insect production unreliable. The latter problem is largely due to periodic contamination of the larval food with harmful moulds, especially *Aspergillus niger* [41,51], and to outbreaks of a granulosis virus disease. Many combinations of antimicrobial agents have been used but none is completely effective. Although large-scale moth production in a near sterile environment may not be economically feasible, surface sterilization of eggs, air filtration and strict attention to sanitation are essential.

The cost of moth production is high because the facilities and equipment required to maintain a clean and controllable environment are expensive, many of the dietary ingredients are very costly, insect yield per unit of diet is low, and rearing procedures are not adequately mechanized. The most expensive dietary ingredient, on the basis of the amount required (ca. 0.7% [55]), is

ascorbic acid, but >50% is lost during and after diet preparation [51]. Some appreciable saving would be realized if a good antioxidant could be found to retard the loss of this essential ingredient. Larval utilization of most diets is low in group rearing (100 g yields ca. 10-20 moths [51,56]), but perhaps a method could be found to destroy the contaminating toxin produced by feeding larvae [57] so that unutilized diet could be reused. Mechanization of procedures of egg collection and sterilization, and for seeding the larval diet with known numbers of eggs, would effect a very appreciable saving in labor.

Codling moth rearing has been centered on production of maximum numbers with little attention to quality despite a growing number of reports on the detrimental effects of prolonged colonization. Labanowski et al. [15], for example, found that laboratory-reared male codling moths mated less often than native males in field cages; this result was confirmed in the field by other investigators [24]. Some reports [24,58] show that prolonged colonization impairs flight, but electrophysiological [59] and sex trap [22] data indicate enhanced olfactory sensitivity in the male. Colonization also may affect the moths' period of activity; the 20th generation of a U.S. colony exhibited the typical morning and evening activity periods, but by the 32nd generation the morning activity period had disappeared [60]. Colonization increases the attractancy and mating frequency of the female moth [15,59]. This enhanced behavior could be detrimental to SIT programs in which both sexes are released since sterile males would likely mate with the more attractive and nearby sterile females before encountering a wild female.

Recent work [46,61] indicates that field performance could be improved by rearing at fluctuating temperatures rather than at a constant high temperature as used in most laboratories. Male moths reared at a minimum temperature of ca. 19°C at night and a maximum temperature of ca. 33°C during the day responded much better to synthetic sex pheromone, to virgin females and to UV light than males reared at ca. 26°C. Male dispersal was also improved. Improved performance was more evident in spring, when temperatures are unfavorable for codling moth activity, than in the heat of summer. The numbers of eupyrene sperm bundles transferred by mated males were about equal for the 2 colonies, but were fewer than those transferred by native males.

Measurement of behavioral changes in codling moth colonies should be conducted primarily in the field with emphasis on male dispersal, olfactory response and mating success. Genetic changes can be monitored electrophoretically [62,63], but it may not be possible to relate these changes to behavior. However,

since most genetic changes are detrimental, sensitive electrophoretic techniques should give advanced warning of behavioral changes that are likely to be undesirable.

Genetic [62] and behavioral [59] differences occur between native codling moth populations. Colonization therefore should be with insects from the area where the SIT is to be used. Colony changes may be delayed in some respects by starting with a fairly large number of native insects so as to ensure adequate numbers of matings in the colony founders. Until good procedures are developed for measuring behavioral changes, it would seem advisable to add native insects to the colony at least once per year or to start a new colony every other year.

2. FIELD CONTROL

Experiments on codling moth control by release of chemosterilized moths are very limited. Release of tepa-treated male moths failed to give adequate control in Washington State [64], but good population suppression was achieved in the U.S.S.R. by release of male moths sterilized with diethylenimide amidothiophosphoric acid [65].

SIT experiments on codling moth control with radiosterilized moths may be divided into (a) release of sterile moths (>90% male and >99% female sterility on the basis of egg hatch) and (b) release of substerile insects.

2.1. Release of sterile moths

The 1st experiment was started in 1962 in a 20-tree abandoned apple orchard in British Columbia [66]. Release of sterile males for 3 consecutive years reduced the overwintering population from ca. 400 larvae to 6. Because sexing was impractical, the next experiment, in a 2-ha abandoned orchard, was with mixed sexes [67]. After 2 years of release the overwintering population was reduced from ca. 5000 larvae to 55 despite some reinfestation from nearby abandoned apple trees.

The success achieved in abandoned orchards led to investigations in commercial orchards. Three Canadian experiments were conducted from 1967-72 [29,68,69]. Some problems arose due to 2 accidental releases of fertile moths and to the occasional failure to maintain the minimum projected ratio of 40 sterile:1 native moth. On the whole, however, control was very much better than in nearby sprayed orchards. The best results were achieved in a 40-ha orchard in which the per cent of codling moth damaged fruit was reduced in 4 years from 0.1 to 0.001 after releasing 11 million

sterile moths [68]. The largest U.S. experiment was conducted in a semi-isolated valley from 1970-72 [49]. In 1970, the population was reduced dramatically by tree removal and insecticides. The following year, insecticide sprays were augmented by sterile moth release and the population declined 92% below that in 1970. However in 1972, despite the release of 1.8 million sterile moths, the percentage of codling moth damaged fruit in 129 of the 168 treated hectares increased to 0.129 from the previous year's low of 0.002. The increased damage was attributed to low ratios of sterile:wild moths in certain areas of the orchard and to behavioral changes in the sterile insects which had been colonized for ca. 15 years [60].

At about this time, U.S.S.R. and Swiss investigators [41,47] showed that the SIT for codling moth control was effective under European conditions, but the abundance of unsprayed fruit trees in many areas will make practical application very difficult.

The effectiveness of sterile males alone (25 krad), females alone (15 krad) and mixed sexes (25 krad) was compared by U.S. workers in 3 sections of a large orchard [60]. In earlier cage tests [25,26,28] the best results were achieved with sterile males, followed closely by sterile mixed sexes, and finally by sterile females. However, in the field experiment there was no appreciable differences between the 3 types of release. If this result is valid, and other field work [46] suggests that it is, release of sterile males in some areas and sterile females in others would greatly reduce the cost of control. But this will not be possible until an effective method of sexing is available.

2.2. Release of substerile insects

Control by 25 krad (85% male and >99% female sterility) and 40 krad (98% male and 100% female sterility) moths was compared in a large British Columbia orchard [70]. After 1 year of release the wild population was reduced ca. 59% in the area with substerile moths and ca. 38% in the area with sterile moths. I had expected to find appreciable superficial fruit damage in the substerile insect release area since previous work [26] had shown that under laboratory conditions many of the progeny of 30 krad males x untreated females feed for a few days before dying. However, this type of damage was virtually absent.

The effectiveness of substerile moth release was examined in more detail by the Swiss [43]. Since they wished to utilize inherited sterility, the irradiation dose was reduced to 10 krad (ca. 60% male sterility) and only male moths were released and only during eclosion of the overwintered insects. Population

suppression was very good in the 1st generation and reasonably good in the 2nd. Two thirds of the F_1 males and one-half of the F_1 females showed a high level of sterility. These and other data indicate that control might be achieved by releasing sub-sterile male moths every other generation.

The Swiss conducted another significant field trial [42] in which diapausing F_1 male larvae (95% sterile) of 10 krad males x untreated females were produced in the laboratory and later exposed in artificial shelters in a small orchard. Emergence of the sterile moths was well synchronized with that of the wild population even though only 1 larval release was made in late winter and 1 in early July. No chemical sprays were needed against the codling moth but the population increased perceptibly in the 2nd year because too few sterile larvae were released. In an adjacent orchard where no releases were made, 2 sprays were required each year. There are many advantages to the release of sterile male larvae, but since the insects must be sexed the immediate outlook is not too bright for this innovative method.

2.3. Commercial feasibility of the SIT

By the early 1970 s it was obvious that the SIT would give good codling moth control but was it economically feasible? To try to answer this question a pilot program was conducted in 320-526 ha of apples and pears in a partially isolated valley in British Columbia from 1976-78 [46]. About 24 000-36 000 sterile moths/ha were released each year. Codling moth damage exceeded the economic threshold (0.5%) in only 1 of 86 treated orchards in 1976, in 6 of 193 orchards in 1977 and in 0 of 157 orchards in 1978. Other results indicated that when populations are brought close to extinction all codling moth control measures can be omitted for 2 or more years depending on degree of immigration. Cost of control was ca. Can. $225/ha per year vs. $95 for chemical control. This cost difference is too great to permit commercial implementation of the SIT in British Columbia at this time.

2.4. Abundance of other pests in sterile moth release orchards

The major change in pest abundance was a reduction of aphids and spider mites to noninjurious levels due to an increase of arthropod predators and parasites. However, the absence of the usual sprays for codling moth control allowed leafrollers to build up eventually to damaging numbers [30,46,64,68].

3. CONCLUSIONS

The codling moth can be controlled very effectively by the SIT but current technology is not sufficiently advanced to permit economic implementation of the method at this time. Cheaper larval diets, better antimicrobial agents, and mechanization of rearing procedures would materially reduce the cost of insect production. Rearing at fluctuating temperatures and better methods of quality control would result in a more competitive insect and in reduced cost of control.

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SIT IN THE CONTROL AND ERADICATION OF *Glossina palpalis gambiensis*

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Abstract

SIT IN THE CONTROL AND ERADICATION OF *Glossina palpalis gambiensis.*

In a five-year research programme in Upper Volta, eradication of *G.p. gambiensis* has been achieved in an area of approximetaly 100 km^2 by the SIT alone as well as by the use of the SIT with one or two applications of non-persistent insecticide before the release of sterile males of this species. Results are presented for the period necessary to obtain an eradication with these different parameters and the additional varying of the release intervals in time and space. The number of sterile males released is presented together with the number of release sessions, and a comparison of costs and efficiency of each release is given.

INTRODUCTION

Field experiments on the genetic control of *G.p. gambiensis* conducted by a combined French – German team in Upper Volta, have resulted in the eradication of this species from a river system of 32 km of aquatic vegetation, which led to its disappearance from an area of more than 100 km^2. The efficiency of the SIT was proven by the release of sterile males alone into a non-modified population, as well as in combination with one or two applications of non-persistent insecticide before beginning the releases.

In these experiments different intervals of releases in time and space were tested to find the most efficient procedure. All experiments led to an eradication of *G.p. gambiensis* after varying periods. An evaluation of each experiment is here presented and an assessment of the procedure that is least costly.

EXPERIMENTAL ZONE

A detailed description of the experimental zone has been given in previous publications so that only a short description is outlined here (Fig.1).

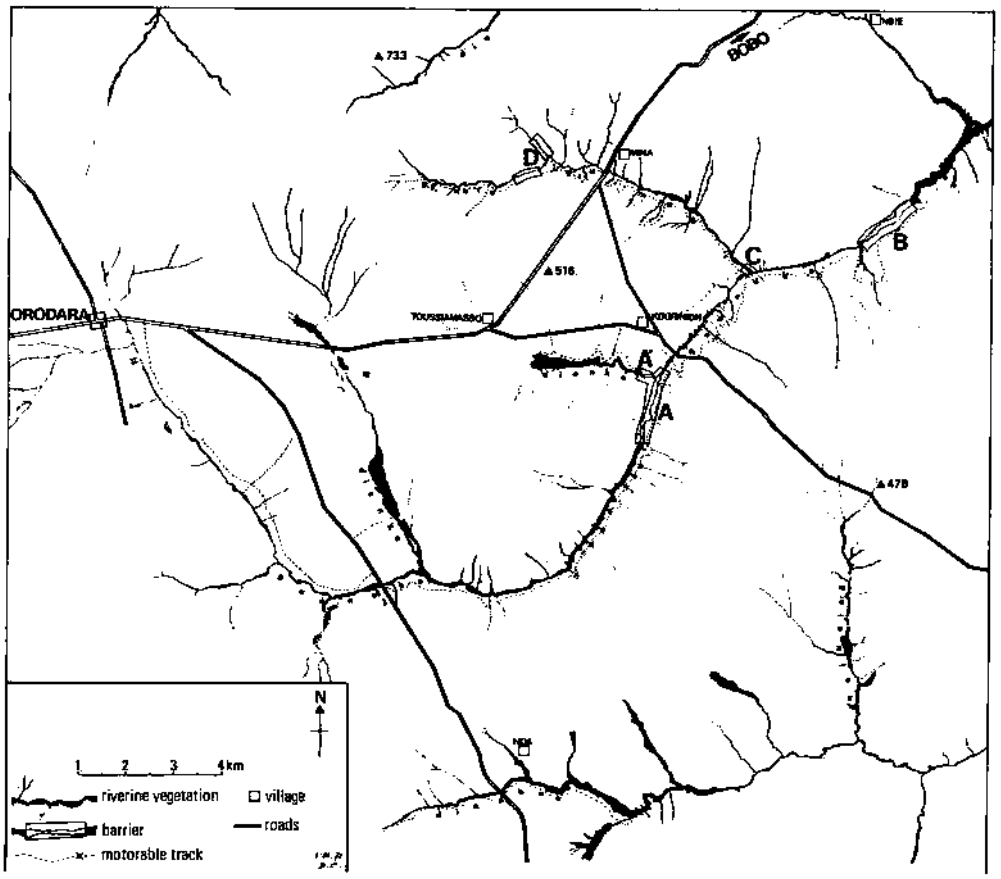

FIG.1. Experimental zone for the sterile male method against G.p. gambiensis (Upper Volta).

The five experimental sections are separated by mechanical barriers (total clearing) and the entire experimental zone is isolated either naturally (heads of rivers) or by a mechanical barrier reinforced by regular spraying of persistent insecticide at the outer limits, and permanent trapping with Challier – Laveissière traps in the barrier itself. The longest barrier measures 1300 m. Two species of *Glossina* are present: *G.p. gambiensis* as target species (95%) and *G. tachinoides* (5%) considered as control.

The lengths of the experimental and the control sections are as follows:

Site I (Guénako) 5.5 km;	on the main river
Site II (Guimpy) 5.5 km;	tributary of the Guénako

Site III (Guimpy north) 2.8 km;	upstream from Guimpy
Site IV (Dienka) 2.2 km;	tributary of the Guénako
Site V (Guénako) 16 km;	16 km of the main river, starting from the source
Site VI (Dienkoa) 5.5 km;	control section; river parallel to Guénako.

STERILIZATION OF MALES

In Upper Volta, with its numerous foci of human sleeping sickness, releases are made of adult sterilized males that have taken their first bloodmeal in the laboratory. Males are sterilized in an irradiator by caesium-137 with a dose rate of 11 krad in a normal atmosphere, producing a sterility level of 95% without causing a significant reduction in the insemination capacity.

CALCULATION OF THE COST OF A STERILE MALE

In the calculation the following elements have been taken into consideration:

buildings (life-span 20 years)
air conditioning
stand-by generator
irradiator (life-span 30 years)
material for the breeding colony
water and electricity
running costs for material, maintenance, etc.
personnel

The latest evaluation resulted in a price of FCFA 54 per sterile male leaving the laboratory.[1] In release sessions FCFA 100 per release per km has to be added for transport as well as the salary for one driver and one man to perform the releases. In our case regular staff was paid overtime for the releases in the afternoons.

PROTOCOL OF THE RELEASES

Sites I and II

After one application of non-persistent insecticide (Thiodan 3.5%) by Swingfog, releases were begun 10 (Site I) and 14 days (Site II) later. Continuous releases were made twice a week to maintain a ratio of sterile males to wild

[1] FCFA = CFA Franc BCEAO; Responsible authority: Banque Centrale des Etats de l'Afrique de l'Ouest.

males of 3 : 1 in Site I, and 7 : 1 in Site II. As the dispersion capacity of the sterilized males of *G.p. gambiensis* was not known, an interval of 200 m between the release points was chosen. The numbers of sterile males released remained constant throughout the whole experiment.

Site III

No application of insecticide was carried out. Releases took place twice a week every 200 m, as in Sites I and II, but this time into a non-modified wild population, where the age structure was not initially changed. Fertile females were present at the time of the first release.

Site IV

One treatment with non-persistent insecticide (Thiodan 3.5% by Swingfog) was carried out before releases, which began in May 1978, twice weekly with one km distance between release points. In order to avoid an overconsumption of sterile males the population density was estimated every month, and the number of sterile males to be released was adapted so that the ratio of sterile males to wild males remained between 7 : 1 and 10 : 1.

Site V

Decamethrin (0.2 g/ha) was applied twice by helicopter, at an interval of 15 days, to bring down the fly population to a manageable level for the production capacity of the colony. Releases of sterile males began the day after the second application, and continued at a rate of two per week. The releases were first carried out only in the middle of this site, that is at point 8 km, to study the speed of dispersion and the longest distances covered. This release point was transferred six weeks later to point 4 km in order to find out if the dispersion was dependent on the peculiarities of the release point. Results from these dispersion trials led to releases every 2 km, ensuring an even dispersion of sterile males over the whole experimental area.

Site VI

Regular controls were carried out to monitor the fluctuation in the annual population.

RESULTS AND EVALUATION OF COSTS

Sites I and II

The population of *G.p. gambiensis* decreased constantly and 12 months later a fall in population density of 70% was found. After 16 months there was eradication of the wild population of *G.p. gambiensis*. During these 16 months, 128 release sessions in Site I and 129 in Site II were carried out. Altogether 90 595 sterile males were released in Site I and 87 654 in Site II. The costs for these sterile males amounted to FCFA 9 625 500. For one release session FCFA 18 000 have been calculated for transport (FCFA 100/km) and the salary for one driver and one capturer for 6 h, FCFA 1620. The costs for 129 release sessions amounted to FCFA 2 530 980 which together with the costs for the flies adds up to a total cost of FCFA 12 156 480 to eradicate *G.p. gambiensis* from 11 km of riverine vegetation, or FCFA 1 105 134 per km of the main river.

Release operations in Sites III, IV and V took place at the same time. Each release covered 22 km of river and the costs per release operation amounted to FCFA 21 620. In the following calculations this sum is divided by the number of kilometres covered in each site and multiplied by the number of releases to arrive at the respective costs of the releases per site.

Site III

Population density dropped constantly during the following dry season, interrupted only by a slight increase during the following rainy season. In October 1979 the wild population of *G.p. gambiensis* became extinct, continued capture sessions confirming this result. To achieve eradication, 228 releases of a total of 64 797 sterile males over a period of 26 months were necessary. The costs for these sterile males were FCFA 3 499 038 and the costs for the release operations were calculated at FCFA 656 868. The total costs amounted to FCFA 4 155 906 or FCFA 1 484 251 per km of the main river.

Site IV

Beginning in May 1978, the decrease in population density reached 70% after only seven months, compared with the same reduction in Site III after 14 months. The wild population became extinct after 19 months compared with 24 months in Site III. These observations demonstrate clearly the benefit of associating treatment with insecticide with the release of sterilized males.

TABLE I. COMPARISON OF COSTS[a]

Site	I	II	III	IV	V	Average value
Treatment before release	Groundspray (Swingfog) Thiodan 3.5%	Groundspray (Swingfog) Thiodan 3.5%	No insecticide	Groundspray (Swingfog) Thiodan 3.5%	Decamethrin by helicopter 0.2 g/ha 2 X	
Initial true density	1 600	600	430	130	4 000	
Number of releases	128	129	228	157	198	
Costs of ♂/km	889 488	860 602	1 249 656	1 165 246	1 215 626	1 076 123
Release costs/km	230 089	230 089	234 595	161 495	203 742	212 002
Total costs/km	1 119 577	1 090 691	1 484 251	1 326 741	1 419 368	1 288 125
Costs of ♂ in percentage of total costs	79.5%	78.9%	84.2%	87.8%	85.6%	83.2%

[a] Total costs for 32 km = FCFA 41 220 000 = FCFA 412 200/km^2.

In 157 release operations 47 473 sterile males were released at a cost of FCFA 2 563 542. The costs for the releases (transport and personnel) amounted to FCFA 355 291, resulting in a total of FCFA 2 918 833 for this site, or FCFA 1 326 740 per km of the main river.

Site V

As the initial release arrangement did not result in an even dispersion of sterile males in this site, only the releases from the date they were carried out at an interval of 2 km are considered. At this time the population of *G.p. gambiensis* has to be evaluated as one with no previous insecticidal treatment, and where fertile females of all age groups were present at the time of the first release.

During 25 months a total of 360 190 sterile males were released in 198 release sessions. The price for the sterile males was calculated at FCFA 19 450 026, the costs of the release operations at FCFA 3 259 872. The total costs to eradicate *G.p. gambiensis* from this site thus amounted to FCFA 22 709 898 or FCFA 1 419 369 per km of the main river.

DISCUSSION

Table I shows that the costs for the eradication of *G.p. gambiensis* from 1 km of the main river amounted to a sum between FCFA 1.09 million and FCFA 1.48 million, following the protocol applied. Considering that the eradication from these 32 km of the main river led to the disappearance of *G.p. gambiensis* from an area of 100 km^2, the total costs of the operation have to be divided by this surface, giving a figure of FCFA 412 000 per km^2. In these calculations the price for the application of a treatment with non-persistent insecticide has not been included as it is nearly negligible. To treat 1 km of river by Swingfog, 1.5 litre of Thiodan and labour costs for 2 h are needed, resulting in a price of FCFA 1800 per km of riverine vegetation. With the use of a helicopter, FCFA 5000 (with two swath-widths double this amount) have to be added to the costs for biological control.

Experiments on the 50% survival rate of sterile males indicate that releases once a week or even every 10 days are sufficient to maintain the necessary number of sterile males in the wild population. This can reduce costs for the releases to one half or even one third. Nevertheless, the bulk of the costs (80%) is spent in the production of sterile males. It has to be emphasized that the sterile male project in Bobo Dioulasso was a research project, and not one with industrialized production. If, by industrialization, the costs for the sterile males can be substantially reduced, reduction of the total cost of the releases can be

more easily gained. However, it should be obvious that even with a reduction in the costs of production (by industrialization) to a quarter of their present costs, the costs of production will still amount to 50% of the total cost of eradication. Consequently the SIT will be most suitable for areas of very low density, or to eradicate flies remaining after other control methods.

CONCLUSIONS

A reduction of costs in the production of the sterile males is essential. Apart from that imperative the following conclusions can be drawn:

The number of sterile males to be released can be considerably reduced by one or two treatments with non-persistent insecticide.

The time necessary to achieve eradication is shorter after a treatment with non-persistent insecticide, thus reducing the release costs.

Further reduction of costs can be achieved by spacing the release intervals at 10 days and releasing every 2 km by bicycle or motor bike, starting from a central point of the release area to which the sterile males have been brought by car.

A reduction in the fly populations at high density spots by traps or screens impregnated with insecticide can additionally reduce the number of sterile males required.

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THE STERILE INSECT TECHNIQUE IN INTEGRATED PEST MANAGEMENT PROGRAMMES FOR THE CONTROL OF STABLE FLIES AND HORN FLIES

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Abstract

THE STERILE INSECT TECHNIQUE IN INTEGRATED PEST MANAGEMENT PROGRAMMES FOR THE CONTROL OF STABLE FLIES AND HORN FLIES.

Two large integrated pest management programmes involving the control of horn flies, *Haematobia irritans* L., and stable flies, *Stomoxys calcitrans* L., have been carried out by U.S. Department of Agriculture scientists. The horn fly project was conducted on the island of Molokai, Hawaii. Its aims were to control the fly population by the insect growth regulator (IGR) methoprene (isopropyl (*E,E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), which was metered into the drinking water of the cattle, and the release of sterile insects. The flies were released daily after exposing the young adults to 2.5 kR. There was excellent survival of the released flies. The release of sterile flies was discontinued after week 21 and no flies were observed on the cattle after week 23. No horn fly larvae could be found in any manure pats in the field after week 16. Horn-fly-infested cattle were moved into the area on week 30 and the fly population increased so the methoprene treatment was terminated on week 39. By week 44 the fly population had returned to normal. The stable fly population was suppressed on the island of St. Croix using sterile insect releases plus conventional control techniques. These included toxic traps, larviciding breeding sites and the release of parasites. The sterile insects, about 1×10^5/day, were released at 2 km intervals over the 218 km^2 island for an 18-month period. For the last six months of the study more than 99.9% of the wild flies were eliminated from the island. However, a few fertile flies were found throughout the study. These fertile flies either came from small isolated breeding sites in the urban areas where no sterile flies were released, or were carried in by boats or planes with domestic livestock from the other islands in the area.

A new approach to insect control came into being with the successful eradication of the screwworm fly from the island of Curacao by the release of sterile insects [1]. The sterile insect technique was quickly adopted by scientists throughout the world and some type of sterile insect research has been carried out on most of the major arthropod pests. However, almost all of this research, especially with livestock pests, has never extended beyond the basic laboratory stage. The reason for this is very simple. We do not know enough about the field biology and ecology of the target species, especially regarding male behavior. We repeatedly underestimate the natural dispersal and recovery rates of wild populations. Also, we are just now beginning to understand the genetic changes that take place in a species that is subjected to mass production for long periods of time. As we understand the field biology and ecology of a target pest, especially one in which the adults are the problem, then the only practical approach for SIT is to integrate it with other control measures. This is especially true with livestock pests such as blood-feeding flies where large releases of laboratory-reared flies would aggravate an already serious problem. Integrated pest management programs work best when control pressures can be exerted on all the life stages of the pest.

Two such integrated pest management programs using sterile insects to control livestock pests have been conducted in the United States. One of these studies was carried out on a portion of the island of Molokai, Hawaii, and the other on the island of St. Croix, U.S. Virgin Islands.

The experiment on the island of Molokai was undertaken by the USDA, ARS, Livestock Insects Laboratory, Kerrville, Texas, to suppress the horn fly, *Haematobia irritans* L. Earlier this same group had demonstrated the feasibility of suppressing a population with the sterile insect technique in a small field study [2]. The objective of the study on Molokai was to suppress the horn fly population in the larval stage using an insect growth regulator (IGR) and then further suppress, and possibly eliminate, the fly population by the release of sterile insects [3]. The study was conducted on the Puu-O-Hoku Ranch which had approximately 750 cattle on 3 000 acres of improved pastures. The ranch was fairly isolated and the prevailing winds were such that there was no movement of flies into the area. This observation was based on marked released studies. Approximately 150 additional cattle present on the ranch were not included in the study because they were pastured in areas of free-flowing streams where the IGR could not be used. Methoprene (isopropyl (*E,E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) was used as the IGR. It was metered into the cattle's drinking water via a tablet formulation [4]. Methoprene, as a feed-through in the water, prevented horn fly larval development in the manure of the treated animals. A concentration of 0.05-0.1 ppm of

the chemical was maintained in the water which was adequate to prevent most larval development of the native horn flies. In earlier studies on Molokai in which cattle had been given water treated with methoprene, the number of horn flies on the animals over a six-week period was reduced ca. 98% (from 360 to 7 flies per animal). Simultaneously with the methoprene application to the drinking water, sterile insects were released.

The flies were imported from two colonies, primarily from the one at Kerrville, Texas, and another in Hawaii on the island of Oahu. Both colonies were maintained using the techniques described by Miller et al. [5]. The adult flies were allowed to feed freely on stanchioned bulls fed alfalfa cubes and coastal Bermuda grass hay; each animal produced ca. 14 kg of feces per day. The female flies would oviposit in the fresh manure. After holding the manure for 24 h for larval hatch, it was mixed with water and artificial media (bagasse, wheat flour, fishmeal and $NaCO_3$). The ratio of manure, water and artificial media was 10:10:3. The larval media was held in corrugated cardboard boxes. This had the advantage that rearing trays did not need to be sterilized and the porous boxes absorbed excess water and allowed fermentation gases to escape and heat to dissipate. The boxes of media were stored on slatted shelving so that air could circulate around them. The holding room was maintained at 27±1°C and 65±5% relative humidity. The pupal incubation chambers were dark except for a 10-cm exit port leading to a lighted, cool room and where emerging flies were trapped in a collecting device. The adult flies, 24-h-old or less, were sterilized by 2.5 kR either in ^{137}Cs source in Kerrville or ^{60}Co in Hawaii. All the released flies were marked with fluorescent dye. Following sterilization the chilled flies were placed in lots of 10 000-12 000 in perforated paper bags containing crumpled tissue paper for resting. These bags of flies were placed in a two-compartmented, insulated container [6] with a frozen eutectic material placed in the top section to keep the flies immobile. A container of soda lime (a CO_2 absorber) was added at the rate of 1 g/1 000 flies. The air in the sealed container was displaced by pure oxygen. The time interval from packaging in Texas to releasing in Hawaii was ca. 30-36 h. The time interval from Oahu to Molokai was ca. 16 h. Greater than 80% of the flies survived the sterilization, marking, shipping and release and were eventually dispersed into the environment. The flies were released daily in the vicinity of cattle at a targeted rate of 1.6×10^6/wk. The number of flies actually released was dependent upon the number of flies available, the degree of sterility in the eggs collected in the wild and the number of flies on the cattle.

The effectiveness of the program was assayed by the number of adult horn flies on each animal and from the numbers and fertility of horn fly eggs collected from fresh manure pats in the field. This study continued for ca. 45 weeks. The methoprene treatment of the drinking water started first, followed a week later by the release of the sterile flies.

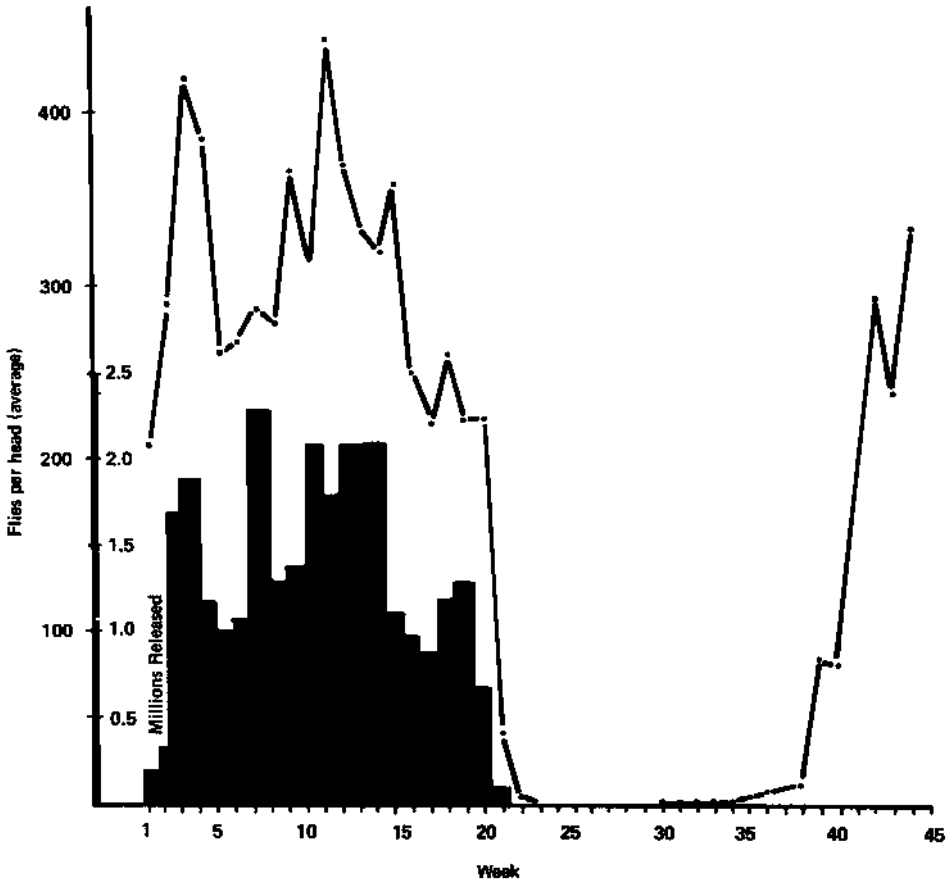

FIG.1. Number of sterile horn flies released per week and number of flies per head of cattle [3].

The population of adult flies averaged 300 per animal for the first 14 weeks then plateaued at ca. 200 until week 20, then rapidly declined to 0 by week 24 (Fig. 1). Thus most of the flies observed, especially after week 10, were probably released flies. There was a steady decline in the number and fertility of eggs in the manure pats (Fig. 2). Initially, each pat averaged 41 eggs with 98% hatch, but by week 14 each pat averaged 0.4 eggs and none were fertile. On week 14 the releases were curtailed to 8×10^5 sterile flies. By week 16 no horn fly larvae were found in any manure pats, indicating complete suppression of the native population in the test area. All the releases were discontinued after the 21st week. No fly eggs were found

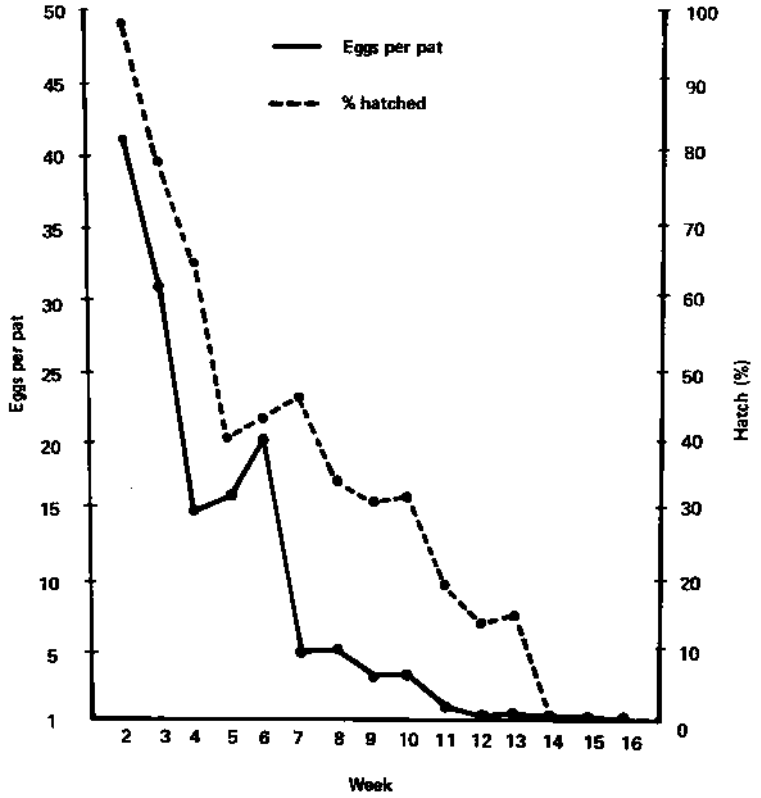

FIG.2. Effect of using methoprene plus sterile insect releases on production and fertility of wild horn fly eggs.

nor adults observed for nine weeks after the last release. By accident, on week 30, horn-fly-infested cattle were moved into the test area and the wild population was reestablished. The methoprene treatment was discontinued on week 39 and by week 44 the adult horn fly population had returned to its pretreatment level. This experiment carried out by the scientists of the USDA Livestock Insects Laboratory, Kerrville, Texas, clearly demonstrated the necessity of SIT in an integrated program for horn flies if eradication is an ultimate objective. Because of the success of this study the program was to be expanded to include the entire island of Molokai (ca. 67 300 ha). Methoprene treatments reduced the horn fly population by ca. 83% ranging from 80-99.8% [7] over the entire island. However, the mass rearing of horn flies at both Texas and Hawaii had to be curtailed and subsequently discontinued, and therefore no releases of

sterile insects were started. In both horn fly colonies there were repeated invasions of arthropods (mites), nematodes and finally the bacteria *Bacillus thuringiensis*. The latter devastated the colonies making it impossible to mass produce the flies. Therefore, these rearing problems must be overcome prior to undertaking another mass release of sterile insects in an IPM program to suppress horn flies.

The control of stable flies (*Stomoxys calcitrans* L.) using an IPM approach with emphasis on the sterile insect technique was undertaken by scientists at the USDA, ARS, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida. St. Croix, U.S. Virgin Islands,was chosen as the test site because of its isolation, variation in topography and size of its native stable fly population. The island's 218 km^2 supports ca. 10 000 cattle and horses plus numerous herds of goats, sheep and swine. There were ca. 100 large stable fly breeding areas mainly at the dairies and beef ranches where silage was being fed. These areas plus numerous small sites produced a stable fly population of ca. 1×10^6 in the wet season and maintained a population of 2×10^5 in the dry season. The rate of increase per generation in the wet season was seldom more than 2X and decreased to 1.2X or less during the dry season. The daily emergence rates, based on adult emergence as well as on the daily loss rates (DLR) derived from mark-release-recapture studies in static populations, indicated that the daily emergence population makes up ca. 25% of the total adult stable fly population.

During the first year of the program the native fly population was surveyed, breeding habitats were determined and control measures developed. During this time the local strain of flies was colonized and mass rearing, sterilization and release techniques were worked out. The rearing facilities had to be designed to prevent any escaping flies because the flies were being reared in the location where the wild flies were to be suppressed and eventually eradicated. Mass rearing was limited because of the size of the facilities. Daily adult production ranged from $2.5\text{-}3.0 \times 10^5$/ day. There was ca. a 30% efficiency from egg to adult. All the flies were reared according to methods developed at this laboratory [8,9]. Approximately 70 000 adults per day were needed to maintain the stock colony. The remaining flies were irradiated and later released in the field. Both males and females were released following exposure of 24-to 48-h-old adults to 2.5 kR of gamma rays from a ^{60}Co source. This dosage yielded 100% sterility in the females and 98±2% sterility in males. Following irradiation these adults were vigorous and highly competitive with the native strain. Studies [10] indicated a 5% reduction in fly efficiency caused by the irradiation. All the flies that were sterilized and released on St. Croix were marked with fluorescent dusts. The dust increased the DLR of the flies by ca. 10%. We felt marking was essential, since we

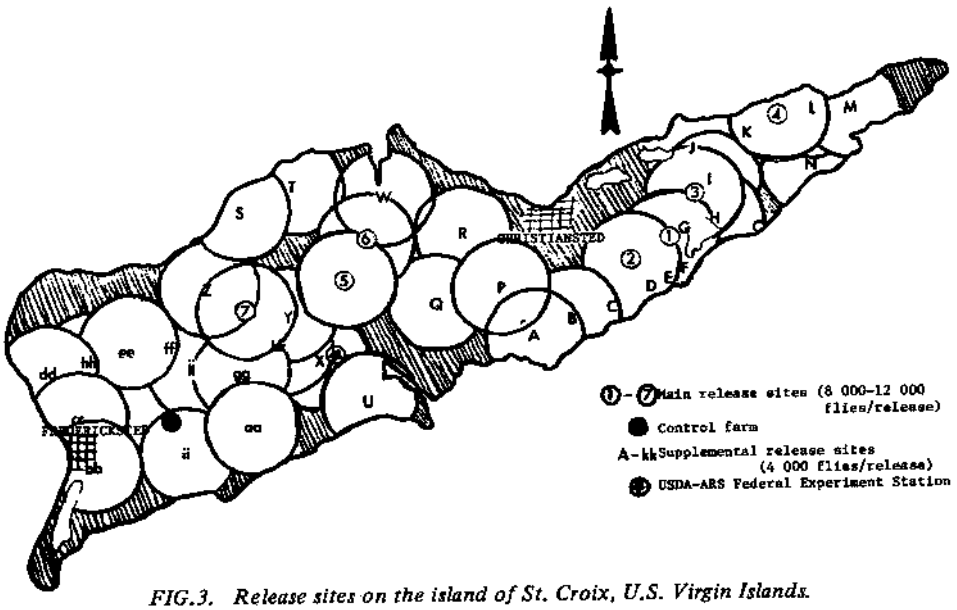

FIG.3. Release sites on the island of St. Croix, U.S. Virgin Islands.

could rapidly derive the field ratio of sterile to normal males found in animal-baited traps [11] located throughout the island, and assay only wild females (unmarked) for sterility. Based on laboratory and field cage studies, the marked sterile flies appeared 15% less competitive than normal flies. However, in the field the sterilized flies exhibited less competitiveness than observed in the field cages. This decreased level of competitiveness was probably caused by the flies being released in areas where they had to migrate long distances to get a blood meal or to mix with the wild-fly population rather than a true loss of competitiveness caused by any physical damage to the flies.

The sterilized flies were released at 2 km intervals throughout the island (Fig. 3) except in the densely populated areas. Because of inaccessibility due to lack of roads, releases were not conducted in areas of the rain forest and a salt marsh on the eastern end of the island. The sterile flies were released in lots of 4 000 from disposable paper bags from a vehicle [12]. At the major dairies and feed lots, 12 000 sterile flies were released near the animal-holding pens. The released flies had a tendency to orient toward the herds of cattle or horses and did not disperse evenly throughout the island.

When the study was started, only one farm was growing and feeding sorghum. Each subsequent year the number of acres planted in sorghum increased, and there was a corresponding increase in the breeding sites and in the number of wild stable flies.

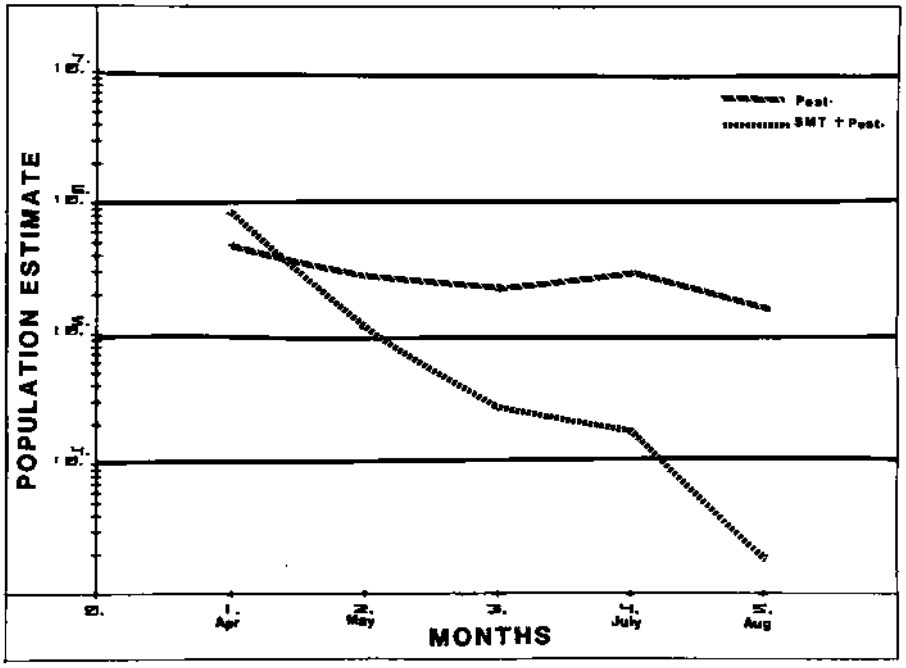

FIG.4. Estimate of stable fly populations on St. Croix using pesticides alone versus pesticides plus release of sterile insects.

Sorghum mixed with animal wastes is an excellent breeding media for stable flies [13]. Since the production of stable flies for release could not be increased because of limited staff and facilities, we reduced the wild-fly population by using methoxychlor to treat some of the larval habitats created by sorghum feeding. The 1% spray acted mainly as an adulticide to the newly emerging adults and ovipositing females. The larval sites were treated routinely starting about three months prior to the systematic releases of sterile flies. These treatments caused an estimated 50-60% overall reduction in the fly populations in the areas. This estimate is based on the fact that although the methoxychlor sprays were used at the major breeding sites throughout the island, the sterile insects were released on only the eastern half (ca. 58 km^2) for a 4-month period. Therefore the effect of the chemical alone and that of the chemical and the sterile insect releases (Fig. 4) could be calculated. The data clearly indicate that the spraying stopped all population growth but that other control methods such as the release of sterile insects were needed to cause a downward trend in the population.

Since the use of methoxychlor sprays or release of sterile flies was not allowed at some breeding sites, other control measures were used. Although cultural control is the preferred method of fly population reduction, few farmers cooperated. Some were persuaded to feed silage from troughs rather than from the ground, and in one instance where a farmer forbade the use of pesticides, all waste material was accumulated at a central point and composted, thereby reducing fly breeding. The parasite *Spalangia endius* Walker was used at a poultry operation of ca. 20 000 chickens to control both the house fly *Musca domestica* L. and stable flies which were breeding in the poultry manure. Following releases of ca. 2.5×10^5 parasites per week for approximately eight months we eliminated both the house flies and the stable flies from this farm. During the last six months of the study an additional control measure was introduced. An attractant toxicant trap system [14] was used at several farms to eliminate the few remaining stable flies. Unfortunately, we did not have sufficient traps or manpower to cover all the isolated and urban breeding sites where (SIT) releases could not be conducted because of concentrated human populations.

The effectiveness of the integrated pest management program for stable fly control was evaluated by two methods. One was an estimate of the total wild stable fly population based on the number observed feeding on the animals and corrected for the sterile to normal ratio observed in the field.[1] The other technique was the degree of sterility in the wild females.

As stated previously, the integrated system was started on the eastern half of the island (58 km^2) in March of 1976. Sterility increased steadily until August in the wild population, as shown in Fig. 5. At that time the fly population was at such a low level that releases on the western half of the island (ca. 83 cm^2) were initiated, and only enough sterile flies were released on the eastern half to maintain population suppression. However, because of a shipping strike we had to use a bran that was milled differently for the larval diet which caused curtailment of fly production and only a few flies could be released. We terminated regular releases on the western half of the island and tried to maintain releases on the eastern half. Still, sterility declined to 70% in November with a corresponding increase in the wild population as shown in Fig. 5. Once the colony fly production returned to normal and sufficient flies were again available for release, sterility on the eastern side rose again to 99% followed by a steady decline in the wild population. Throughout this period sterility on the western

[1] At weekly intervals, 'animal counts' were made and heifer-baited traps were set at ten farms. Consequently, the number of flies observed on an animal could be amended to derive only the wild flies feeding and the total wild stable fly population could be estimated. Since all released flies were marked, the ratio of released to wild could be determined.

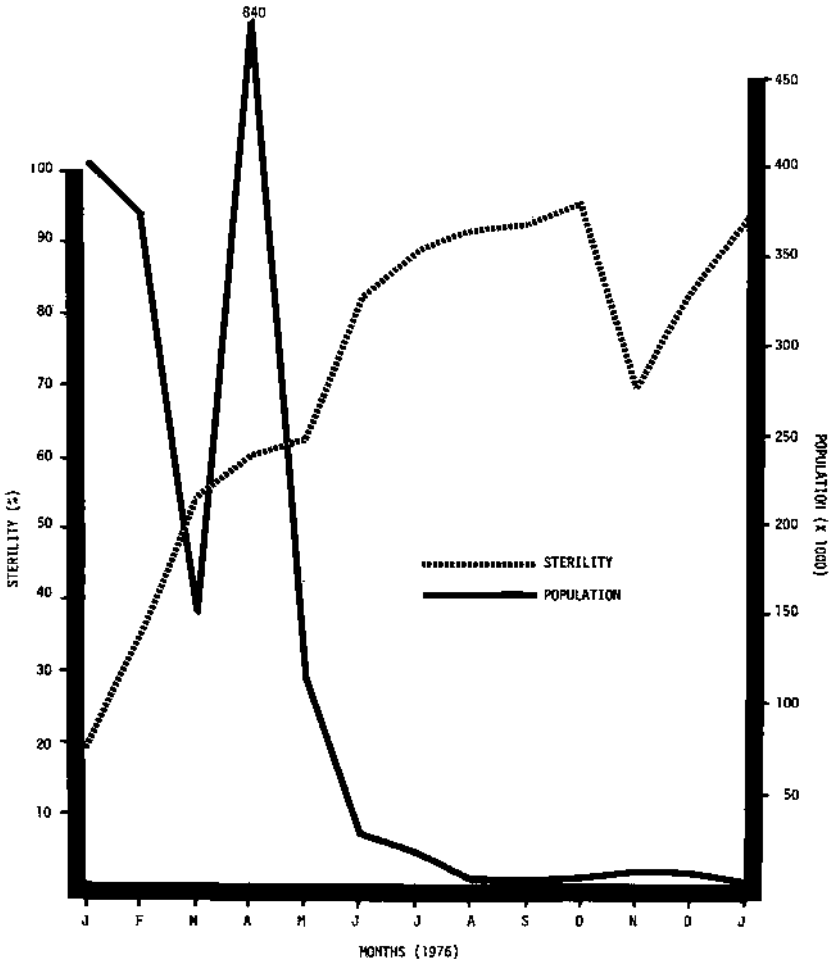

FIG.5. Effect of IPM on wild stable fly fertility and populations on St. Croix.

half of the island remained at the same level and the population did not decrease until sterile insects were again released in sufficient numbers. The larvicide treatments used throughout this period helped curtail the rapid buildup of the population.

By January 1977 all systems of the IPM program were functional. The wild-fly population rapidly declined to an estimated low level of only 350 wild stable flies over the entire island. Sterility likewise rose and remained at ca. 99+%. The wild-fly population was so low that frequently weeks elapsed before a wild female could be captured for sterility assessment.

If it was fertile, it could not be determined whether it came from some small isolated breeding area, was indigenous to St. Croix, or was introduced with livestock imported from the other islands.

The study proves that an IPM program using sterile insects could successfully control stable flies over a large area. It is possible to obtain total population control by the release of sterile males alone, but the use of integrated control techniques is a more practical approach. Source reduction, insecticide treatments of some of the larval breeding sites, parasite releases and a toxic trap were some of the controls that were incorporated into this IPM program along with sterile insect release.

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LEPIDOPTERAN MASS REARING

An inside view

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Abstract

LEPIDOPTERAN MASS REARING: AN INSIDE VIEW.

Major operations for mass rearing Lepidoptera are reviewed from the control room of a facility including metering of dietary ingredients, diet preparation, egg treatment and placement on diet, larval development, adult colony maintenance, pupal harvest, distribution, acquisition and storage. The first three operations are performed in maximum security areas with filtered air and strict control of contamination, The following two occur in less isolated places but have more precisely regulated temperature, relative humidity, light quality and photoperiod, and air circulation. In addition, adult colonies are confined so that their wing scales and other potential human allergens can be collected in filters. A microprocessor-based data acquisition and environmental control system monitors and regulates all the production and air-conditioning equipment. It is an essential part of the quality control subsystem designed to provide continuous feedback for process control, a procedure for periodically sampling insects along the production line at progressive stages and ensuring that the means and variability of their measured characteristics are within specified tolerances. A description follows of the responsibility of production, quality control and managerial units in the dynamics of maintaining viable colonies.

After more than 25 years of diligent research, we have the capability to rear massive quantities of a wide variety of lepidopteran pests (Table I). Moreover, we can do so dependably and with increasing assurance that they will be behaviorally competitive. Current emphasis is on rearing them less expensively, so they can be used more ubiquitously for insect pest management. This objective is requiring innovative adaptation of advanced technology developed for other industries and the application of sound management practices. Inevitably, contemporary research-oriented rearing will continue to evolve into practical, commercially feasible production.

TABLE I. LEPIDOPTERA THAT COULD BE MASS-REARED USING EXISTING PROCEDURES THAT PROVIDE ARTIFICIAL OVIPOSITION SUBSTRATES AND ESTABLISH LARVAE ON ARTIFICIAL DIET WITHOUT SUBSEQUENT HANDLING PRIOR TO PUPATION

Arctiidae
- Estigmene acrea (Drury) - saltmarsh caterpillar

Bombycidae
- Bombyx mori (L.) - silkworm

Gelechiidae
- Pectinophora gossypiella (Saunders) - pink bollworm
- Phthorimaea operculella (Zeller) - potato tuberworm
- Sitotroga cerealella (Olivier) - Angoumois grain moth

Geometridae
- Operophtera brumata (L.) - winter moth

Lasiocampidae
- Malacosoma disstria Hubner - forest tent caterpillar

Lymantriidae
- Lymantria dispar (L.) - gypsy moth

Megalopygidae
- Megalopyge opercularis (J. E. Smith) - puss caterpillar

Noctuidae
- Agrotis ipsilon (Hufnagel) - black cutworm
- Agrotis orthogonia Morrison - pale western cutworm
- Anagrapha falcifera (Kirby) - celery looper
- Anticarsia gemmatalis Hubner - velvetbean caterpillar
- Autographa californica (Speyer) - alfalfa looper
- Euxoa auxiliaris (Grote) - army cutworm
- Feltia subterranea (F.) - granulate cutworm
- Heliothis armigera (Hubner) - American bollworm
- Heliothis virescens (F.) - tobacco budworm
- Heliothis zea (Boddie) - corn earworm
- Mamestra configurata Walker - bertha armyworm
- Peridroma saucia (Hubner) - variegated cutworm
- Pseudaletia unipuncta (Haworth) - armyworm
- Pseudoplusia includens (Walker) - soybean looper
- Spodoptera exigua (Hubner) - beet armyworm
- Spodoptera frugiperda (J. E. Smith) - fall armyworm
- Spodoptera littoralis (Boisduval) - cotton leafworm
- Spodoptera praefica (Grote) - western yellowstriped armyworm
- Trichoplusia ni (Hubner) - cabbage looper

Oleuthreutidae
- Argyroploce leucotreta Meyrick - false codling moth
- Endopiza viteana Clemens - grape berry moth
- Grapholitha molesta (Busck) - Oriental fruit moth
- Gretchena bolliana (Slingerland) - pecan bud moth
- Laspeyresia pomonella (L.) - codling moth
- Rhyacionia buoliana (Schiffermuller) - European pine shoot moth

Pieridae
- Colias eurytheme Boisduval - alfalfa caterpillar
- Pieris rapae (L.) - imported cabbageworm

Pyralidae
- Achroia grisella (F.) - lesser wax moth
- Amyelois transitella (Walker) - navel orangeworm
- Anagasta kuehniella (Zeller) - Mediterranean flour moth
- Cadra figulilella (Gregson) - raisin moth
- Chilo suppressalis (Walker) - Asiatic rice borer
- Chilo zonellus Swinhoe - sorghum stemborer
- Diatraea grandiosella (Dyar) - southwestern corn borer
- Diatraea saccharalis (F.) - sugarcane borer
- Ectomyelois ceratoniae (Zeller) - carob moth
- Elasmopalpus lignosellus (Zeller) - lesser cornstalk borer
- Ephestia cautella (Walker) - almond moth
- Ephestia elutella (Hubner) - tobacco moth
- Galleria mellonella (L.) - greater wax moth
- Ostrinia nubilalis (Hubner) - European corn borer
- Plodia interpunctella (Hubner) - Indian meal moth
- Vitula edmandsae serratilineella Ragonot - driedfruit moth

Saturniidae
- Hyalophora cecropia (L.) - cecropia moth

Sesiidae
- Synanthedon pictipes (Grote & Robinson) - lesser peachtree borer

Sphingidae
- Manduca sexta (L.) - tobacco hornworm

Tineidae
- Tineola bisselliella (Hummel) - webbing clothes moth

Tortricidae
- Adoxophyes orana Fischer von Roslerstamm - smaller tea tortrix
- Argyrotaenia velutinana (Walker) - redbanded leafroller
- Epiphyas postvittana (Walker) - light-brown apple moth
- Platynota stultana Walsingham - omnivorous leafroller

The focal point for any lepidopteran mass-rearing system, indeed the definitive characteristic of large versus small scale rearing, is the integrated facility. This realization prompted Finney and Fisher [1] to state their industrial concept of mass production as the 'economical production of millions of beneficial insects' in 'assembly-line style' with the goal being 'to produce with minimum manhours and space the maximum number of fertile females...in as short a time and as inexpensively as possible'. This definition remained essentially unchanged until Mackauer [2] attempted to quantify the biological aspects with 'production per generation cycle of one million times the mean number of offspring per female'. Chambers [3] considered this definition an order of magnitude or more too large for non-entomophagous insects and combined the biological with the economical approach. However, because of the tremendous diversity in the reproductive potential of insects, we define mass production in purely mechanistic terms prescribed by the rearing operations and associated facilities. The basic criteria are systematization (coordination of independent operations), integration (specialized facilities), automation (mechanized equipment) and utilization (support for outside research or control). Thus, mass rearing is a systematic enterprise accomplished with machinery in integrated facilities for the purpose of producing a relatively large surplus of insects for distribution.

Virtually every component of a production system can be analyzed by touring a facility and inspecting the physical location, equipment, and materials associated with each rearing operation. By visualizing where and by what means these operations are performed, pertinent questions can be framed about the arrangement of space; allocation of resources; provisions for sanitation, safety, and comfort; applicability of alternative rearing techniques; potential for automation; and the ultimate effects of this environment on the development and behavior of insects.

Since essentially all existing lepidopteran mass-rearing facilities are unique in design and purpose for which they were constructed, we will describe and analyze a hypothetical system based on successful ones developed for Heliothis species [4], the pink bollworm, Pectinophora gossypiella (Saunders) [5] and the cabbage looper, Trichoplusia ni (Hubner) [6]. The major rearing operations to be reviewed from the operations center will include metering of dietary ingredients, diet preparation, egg treatment and placement on diet, larval development, adult colony maintenance, pupal harvest, product distribution, and materials acquisition and storage. This approach is intended to provide a graphic visualization of the location, appearance and function of each component of an advanced system for mass-rearing Lepidoptera.

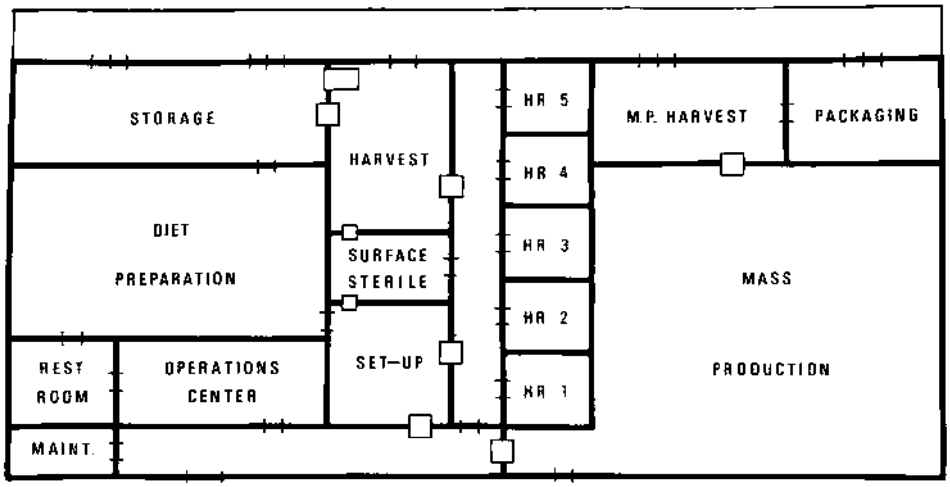

FIG.1. Floor plan of a 15 X 33 m facility for mass-rearing Lepidoptera. Containers of diet are wheeled on carts to the set-up room to be provisioned with surface-sterilized eggs and transferred to the holding rooms (HR, 1–5, 39 m^2) or 175 m^2 mass production larval development area. After pupation has been completed the carts are moved to the colony or 46 m^2 mass production (MP) harvest and packaging room (cubicles in walls are pass-throughs, the rectangle between harvest and storage is a pass-through autoclave). The facility has 168 m^2 for storage, diet preparation, surface sterilization, set-up and harvest. The operations center is 22.5 m^2.

Facilities and Operations

Our hypothetical facility has 500 m^2 of floor space, enough to produce 500 000 pupae per day of one species (Fig. 1). Design criteria were operational efficiency, economy, sanitation, flexibility and durability. Building materials and construction methods were similar to those described by Griffin [7] and Owens [8], predicated on facilities for rearing the boll weevil, Anthonomus grandis Boheman [9], and the sugarcane borer, Diatraea saccharalis (F.) [10]. Large quarantine centers also provided useful information on materials and design [11], [12], [13]. Continuity of work and prevention of frequent contamination are assured by controlling access. Furthermore, internal traffic flows from more to less clean areas, and the recycling of materials is made possible by steam or chemical sterilization. Procedures for preventing or eliminating insect pathogens were adopted from Sikorowski [14], [15].

The diet preparation area can be viewed from the operations center through a safety-glass wall and access is via a double door pass-through (one locks while the other is open). This isolated

room has electrostatically filtered air (or 99.97% efficient particulate air filters), and devices for preparing, flash-sterilizing and dispensing artificial diet. An exhaust hood removes dust particles and fumes produced during diet preparation. Space is available to store enough supplies for one day's production and to arrange mobile racks of larval rearing containers to receive diet. Cleaning is facilitated by the durable and uncluttered surfaces in the room and on the equipment, the divided wash basin and the closable floor drain. The entire room, although under positive pressure, can be sealed and fumigated.

Although a variety of artificial diets have been developed for Lepidoptera [16], mass rearing requires that they be as economical, simple and uniform as possible. Initially, these diets were composed of some combination of wheat germ, casein, yeast, ground beans, and processed beans, seeds or plants (i.e., soybean flour, cottonseed meal and alfalfa meal) mixed with vegetable bulk, vitamins, antimicrobials and agar. Subsequent efforts to reduce the cost of ingredients and preparation have focused on alternative protein sources and gelling agents. Thus, CSM (corn, soy flour and milk solids) and WSB (wheat soy blend) have been used successfully for some species [17], [18], and inexpensive industrial gums can be substituted for agar [19], [20]. However, we use a pinto bean-based diet gelled with xanthane and other industrial gums because it satisfies all the basic criteria, may be used for many species, and does not require cooking.

The diet is mixed in a 200-liter steel vessel with a pneumatically operated, high-speed blender that provides adequate shear. A positive displacement pump automatically transfers it to the flash sterilizer and dispenses it into 6-liter plastic containers (Fig. 2).[1] These containers are designed to hold their shape after repeated treatment with heat and sanitizing chemicals, and to prevent the escape of neonate larvae. They also are reasonably priced, readily available, durable and translucent. Removable partitions can be added to isolate developing larvae and facilitate pupal harvest. An adequate evaporation rate is achieved by removing one-third of the surface of the lid and replacing it with a porous material (i.e. polypropylene-Porex® filter) [4]. After these containers are filled, the diet is allowed to cure for a few hours so that formaldehyde and other fumes can evaporate, and gelling can be completed before they are transferred to the set-up room.

The egg surface-sterilization and colony set-up areas are maintained with the same hospital operating-room-like conditions

[1] The U.S. container industry has discontinued production of the waxed paper 'ice cream' cups and paper lids that have proven so useful for rearing Lepidoptera. Their plastic replacements must be modified to be suitable for this purpose.

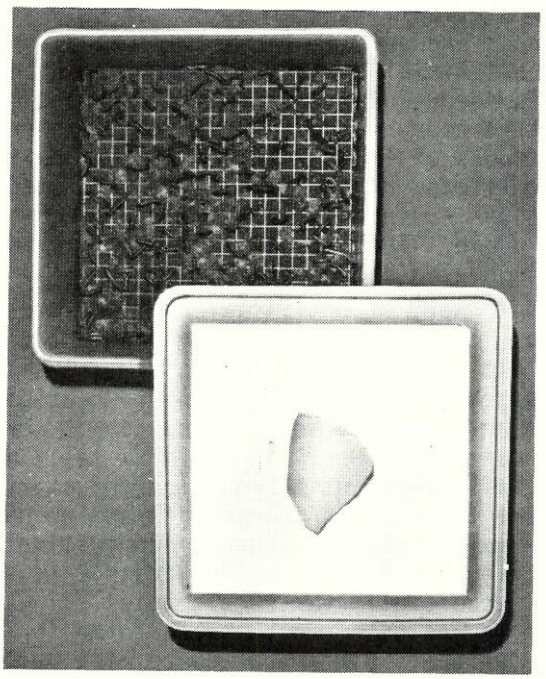

FIG.2. Large plastic container (6-liter) for rearing lepidopteran larvae showing the diet-filled partition, lid with one-third of surface removed and covered with a polypropylene filter, developing larvae, and spent egg sheet attached to the lid.

as the diet preparation room, including limited access, positive pressure, electrostatically filtered air and daily cleaning. Egg-laden cloth, paper, plastic or screen oviposition substrates are collected from the moth colony rooms, transferred to the surface-sterilization room, submerged immediately in a vat of sodium hypochlorite solution, and gently agitated for several minutes. This dissolves the cement that binds eggs to each other and the substrate, and excoriates them to remove surface contamination and accelerate eclosion [21]. This treatment is followed by successive rinses in water and a sodium thiosulfate buffer, and another wash cycle using formalin if cytoplasmic polyhedrosis virus is suspected [5]. Free surface-sterilized eggs are collected by filtration, distributed uniformly in a carbohydrate suspension (i.e. agar, sugar or industrial gums) transferred to the colony set-up room, metered on to container lids or diet, and dried under blowers. Finally, the containers are capped and transported via a double door pass-through to the brood colony (Fig. 1, HR) and mass-production development rooms.

Larval development occurs in a series of small precisely controlled rooms rather than large open areas [22], [23], [24]. This provides more uniform environments and flexibility as production requirements fluctuate. It also promotes cleaning, fumigation and renovation, and prevents the catastrophic loss of an entire production colony if environmental controls fail. Each chamber has an independently regulated radiator connected to a common heater/chiller. Another identical heater/chiller is phased in and out 50% of the time to provide a reserve capability. The unfiltered incoming and recirculating air is exchanged through a blower at a rate of ca. 3 times per minute. All of the chambers have uniform temperature, RH, and lighting (26°C, 55% RH, L:D 14:10 photoperiod typically). The objective is to maintain larvae in an energy efficient way for 40 or more days (if necessary) without handling, feeding or contamination.

Containers with fully developed pupae are wheeled on carts to the respective harvest areas via a one-way pass-through. These rooms are designed to isolate potentially contaminated materials from the remainder of the facility and to provide for the harvesting and cleaning of insect products. An efficient and quiet exhaust system removes airborne particles from the uncapping station and fumes from the cleaning vats. These vats contain chemicals for separating the spent diet, frass and insects, and for preliminary cleaning of container components. The room is durable and contains a minimal amount of equipment so it can be 'flushed out' by means of a large floor drain. Superficially clean pupae are transferred progressively from here to the surface-sterilization and colony set-up rooms. Materials and equipment to be recycled are sterilized and transported to the storage area through a pass-through. The storage room also contains devices for handling, metering and mixing dietary ingredients.

Rooms containing brood moths are identical to larval development rooms except cabinets are installed to house various kinds of adult cages and confine scales and other body parts (Fig. 3).[2] Since airborne particles from colony rooms are potentially hazardous to humans [26], [27] and may transport insect pathogens [5], the cabinets are connected to an industrial cyclone or mechanical dust filter that efficiently removes particles 0.5 μ or larger. Electrostatic filters could be added if necessary. Typically the cage environment is maintained at 25°C and 80% RH with a L:D 14:10 photoperiod. Also, the lighting is broad spectrum with low intensity at night [25].

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[2] Ancillary facilities often are used for larval development and adult colony rooms. Portable or stationary commercial controlled-temperature rooms are suitable for this purpose.

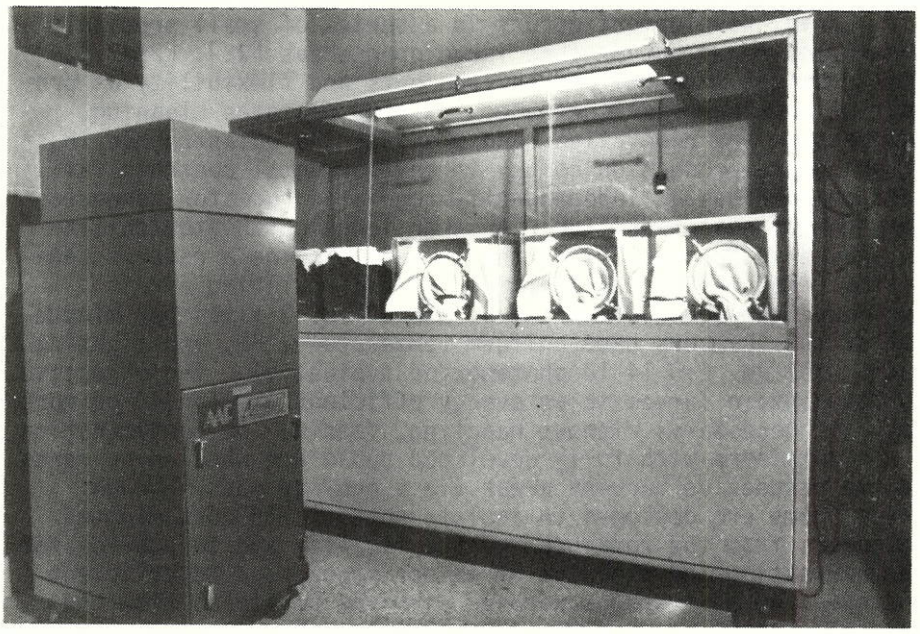

FIG.3. Air filtration system for maintaining moth colonies including a cabinet for holding cages and an industrial dust filter.

Management of Resources and Colonies

The operations center, actually a sophisticated office placed centrally in the facility, is provided with large windows so that most other areas can be viewed from its location. This arrangement and the central intercom facilitate efficient coordination of operations and communication among staff members. There also is a data acquisition system for continually monitoring the temperature and RH of all the rooms. In this center, the insectary manager reviews check sheets that indicate performance of rearing tasks, use of materials, and completion of cleaning and maintenance activities. He also holds meetings to assign and schedule work, train personnel, promote team work, assure health and safety, plan tours, establish budgets, prepare inventories, purchase equipment and supplies, and compile internal and external reports [28].

This center also is the place where decisions are made concerning colony management. Since the insect product must conform to biological standards [3], samples are derived periodically from the production line and life history parameters are measured including % egg hatch, larval size (rate of development), pupal

weight, % adult emergence, mating frequency, longevity, and oviposition rate (viable eggs per mated female). In addition, tests are conducted on adult behavior to determine relative motility, synchrony, orientation ability and competitiveness. Behavioral bioassays used to measure these parameters include a mark-release-recapture grid, an actograph and ratio tests coupled with observations [29]. The results of these life history and behavioral determinations are plotted on process control charts to assure that the insect product remains within tolerance [30].

Methods-improvement research is another important activity that occurs elsewhere but directly affects management decisions made in the operations center. This work usually is performed to solve problems, increase efficiency, or incorporate improved materials and methods. For example, we currently are perfecting a new large-container rearing and associated harvesting system. We also need a less expensive diet, a completely automated egg metering system, and better ways to measure insect performance and assure purposeful rearing. Basically, the cost of labor and materials must be reduced in every possible way to keep mass rearing economically feasible.

We would like to conclude this hypothetical tour by emphasizing the current state of lepidopteran mass-rearing technology. It is quite apparent that we have the immediate capability for mass-producing at least 60 species in support of autocidal and biological insect control practices. Moreover, the utility and economy of this approach to managing insect populations has been proven repeatedly. We must not wait for catastrophic losses due to insecticide resistance before employing this ecologically sound technology. It should be phased in and perfected while alternatives still exist. The IAEA is among a few organizations that have pioneered and guided the massive 25-year effort that has yielded this technology. It is appropriate, then, that IAEA should report its availability and encourage its use on an international scale.

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TSETSE MASS REARING AND THE STERILE INSECT TECHNIQUE

Sessions 3 and 4

ELEVAGE DE *Glossina palpalis gambiensis* EN AFRIQUE

Bilan de six années d'élevage sur animaux nourriciers

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Abstract–Résumé

REARING OF *Glossina palpalis gambiensis* IN AFRICA: SUMMARY OF THE RESULTS OF SIX YEARS OF REARING ON HOST ANIMALS.

Glossina palpalis gambiensis has been reared in Bobo-Dioulasso (Upper Volta) since March 1975 on host animals in two insectaria: rabbits have been used for six years in one and guinea pigs for two years in the other. Altogether 3 643 968 pupae have been produced by these colonies, comprising an average of 30 000 females in one and 12 000 in the other. As a result, 900 000 males were released in the field after sterilization. The principal characteristics of this rearing programme in Africa are given together with the practical implications thereof.

ELEVAGE DE *Glossina palpalis gambiensis* EN AFRIQUE: BILAN DE SIX ANNEES D'ELEVAGE SUR ANIMAUX NOURRICIERS.

Glossina palpalis gambiensis est élevée à Bobo-Dioulasso (Haute-Volta) depuis mars 1975 sur animaux nourriciers dans deux insectariums: l'un utilise le lapin depuis six ans et l'autre a utilisé le cobaye pendant deux ans. Un total de 3 643 968 pupes ont été produites par ces colonies qui représentent 30 000 femelles en moyenne dans l'un et 12 000 femelles dans l'autre. Elles ont permis de lâcher sur le terrain 900 000 mâles après stérilisation. Les principales caractéristiques de cet élevage africain sont données ainsi que les implications pratiques qui en découlent.

1. PRESENTATION

Depuis sa création en 1975, le Centre de recherches sur les trypanosomoses animales (CRTA) de Bobo-Dioulasso a entretenu une colonie de *G.p. gambiensis* sur lapins puis une colonie sur cobayes [1] qui ont assuré les besoins en mâles stériles d'un programme de six années de lâchers sur le terrain. Les performances obtenues en conditions tropicales sont présentées dans cette note. Cet élevage s'est effectué dans deux insectariums indépendants.

FIG. 1. Evolution des effectifs de femelles reproductrices.

L'insectarium I a été mis en service en mars 1975 avec 5333 pupes envoyées de Maisons-Alfort [2], cette souche étant originaire de Haute-Volta. L'effectif de 30 000 femelles a été atteint en avril 1976. Le lapin a constitué l'animal nourricier de cette colonie qui est toujours entretenue au CRTA.

L'insectarium II, construit par mesure de sécurité à la suite de divers accidents, a été mis en fonction en avril 1977 à partir du surplus de la colonie I. L'effectif, de 10 000 à 15 000 femelles, a été nourri sur cobayes jusqu'en juin 1979, puis cet animal a été progressivement abandonné au bénéfice du lapin, jugé plus profitable.

Ces deux colonies ont atteint ensemble, en octobre 1978 et juin 1979, un effectif de 50 000 femelles reproductrices.

De conception très similaire, ces insectariums comprennent chacun une salle d'alimentation précédée d'un sas et une salle de stockage, le tout représentant 270 m^3 pour l'un et 120 m^3 pour l'autre. A l'insectarium I est adjointe, en plus, une salle d'irradiation.

La climatisation a été assurée en 1975 et 1976 par une installation centrale (compresseurs et humidificateurs par vaporisation d'eau) dans l'insectarium I. Ce matériel peu fiable et onéreux a été alors progressivement remplacé par des climatiseurs et des humidificateurs par atomisation de l'eau (modèle «Defensor») qui fonctionnent jusqu'à ce jour avec satisfaction dans les deux insectariums.

Une technicienne expatriée a dirigé les élevages qui ont occupé, dans chaque insectarium, 7 et 4 personnes respectivement à raison de 7 heures de travail par jour pour les manipulations (éclosions, accouplements, séparation, marquage) et pour l'alimentation, ce qui représente une personne pour l'entretien de 5000 femelles reproductrices.

Deux employés effectuent le nettoyage des salles et du petit matériel.

2. RESULTATS

Le bilan général repose sur quatre critères qui ont été particulièrement surveillés durant ces six années:

- les effectifs de femelles reproductrices;
- la productivité de ces femelles;
- le pourcentage d'éclosions;
- la mortalité des femelles.

Un seuil critique a été fixé pour chacun d'eux qui constitue un signal d'alarme lorsqu'il est dépassé.

2.1. Effectifs des femelles reproductrices (fig.1 et tab.I)

On peut distinguer deux phases dans chacune des colonies des insectariums I et II.

a) une phase de croissance:

– dans l'insectarium I, celle-ci a permis en 15 mois de passer de 2600 femelles au plafond de 30 à 35 000 femelles;

– dans l'insectarium II, il a fallu 18 mois pour passer de 3000 femelles au plafond de 15 000 à 20 000 femelles malgré des apports de femelles issues de l'insectarium I (8272 en 1978 et 4033 en 1979).

b) une phase stationnaire: les effectifs sont alors maintenus à un niveau constant par élimination des femelles (vieilles de plus de 3 mois ou jeunes écloses) en excédent. Cette mesure est dictée par des impératifs de place et de nombre d'animaux nourriciers disponibles.

Au cours de ces deux phases, on note les accidents suivants qui ont contrarié, soit la croissance, soit le maintien du plafond désiré:

- remise en service trop précoce de lapins traités avec des antibiotiques (pénicilline-streptomycine, 6-7-9 avril 1976) entraînant une forte stérilité et une mortalité élevée;
- refroidissement excessif des femelles au moment du triage;
- pannes du système de climatisation (excès ou manque);
- intoxications par insecticides, soit par introduction directe dans les insectariums, soit indirectement par l'intermédiaire du sang de lapin, ce dernier étant nourri avec des céréales traitées (Dieldrin);
- surexploitation des lapins ou des cobayes (anémie);
- contamination accidentelle par *T. brucei* ayant nécessité à deux reprises la suppression de toutes les colonies de glossines.

En moyenne, il y a dans ces deux insectariums, 24 924 femelles dans l'un et 10 400 femelles dans l'autre.

TABLEAU.I. PRINCIPALES CARACTERISTIQUES DES DEUX COLONIES DE *Glossina palpalis gambiensis* DE 1975 A 1980 A BOBO-DIOULASSO

		1975	1976		1977	
		2è semestre	1er semestre	2è semestre	1er semestre	2è semestre
1) Nombre moyen de femelles par jour	Insectarium I	6895	20 161	20 778	32 333	31 076
	Insectarium II	–	–	–	–	4716
2) Nombre de pupes produites	Insectarium I	83 615	188 825	194 270	370 530	289 118
	Insectarium II	–	–	–	–	50 334
3) Nombre de pupes par femelle par 30 jours	Insectarium I	1,85	1,63	1,45	1,90	1,54
	Insectarium II	–	–	–	–	1,86
4) Pourcentage d'éclosion	Insectarium I	91,43	81,41	89,46	86,13	85,73
	Insectarium II	–	–	–	–	80,80
5) Mortalité journalière des femelles (p.100 du nombre moyen)	Insectarium I	1,28	1,73	1,38	1,41	1,77
	Insectarium II	–	–	–	–	1,82
6) Mortalité des femelles à l'éclosion (p.100)	Insectarium I	1,27	5,19	5,93	12,21	4,61
	Insectarium II	–	–	–	–	4,10
7) Mortalité des femelles avant accouplement (p.100)	Insectarium I	3,37	13,03	7,95	7,62	4,19
	Insectarium II	–	–	–	–	18,85
8) Mortalité des femelles après accouplement (p.100)	Insectarium I	35,72	40,90	33,87	26,88	47,04
	Insectarium II	–	–	–	–	37,44

	1978		1979		1980		Moyenne/ semestre	
	1er semestre	2è semestre	1er semestre	2è semestre	1er semestre	2è semestre		
1)	30 261	31 175	29 853	27 568	30 760	13 304	24 924 ± 5136	
	6146	12 562	14 193	18 151	10 724	6314	10 400 ± 3241	
2)	329 626	355 814	275 987	371 456	340 245	105 030	264 046 ± 63 400	Total 2 904 516
	64 208	138 855	158 245	165 186	113 346	49 278	98 445 ± 38 331	Total 739 452
3)	1,81	1,92	1,85	1,92	1,84	1,39	1,74 ± 0,11	
	1,72	1,86	1,87	1,82	1,65	1,29	1,72 ± 0,14	
4)	80,85	80,18	87,99	84,99	90,11	86,80	85,91 ± 2,28	
	88,71	84,56	89,14	88,13	85,01	81,84	85,45 ±2,52	
5)	1,75	2,07	1,95	1,94	1,07	1,49	1,62 ± 0,18	
	2,26	2,19	2,09	1,48	1,51	1,61	1,85 ± 0,23	
6)	6,00	11,64	6,37	6,27	1,31	1,87	5,69 ± 2,20	
	2,71	2,93	1,10	0,77	2,05	2,13	2,25 ± 0,84	
7)	3,80	11,38	14,34	20,02	4,76	9,73	9,10 ± 3,16	
	21,09	17,58	8,04	4,79	5,86	10,13	12,33 ± 5,04	
8)	45,60	45,27	43,58	38,85	27,62	37,63	38,45 ± 4,18	
	52,53	50,34	56,17	40,01	37,86	42,32	45,23 ± 5,76	

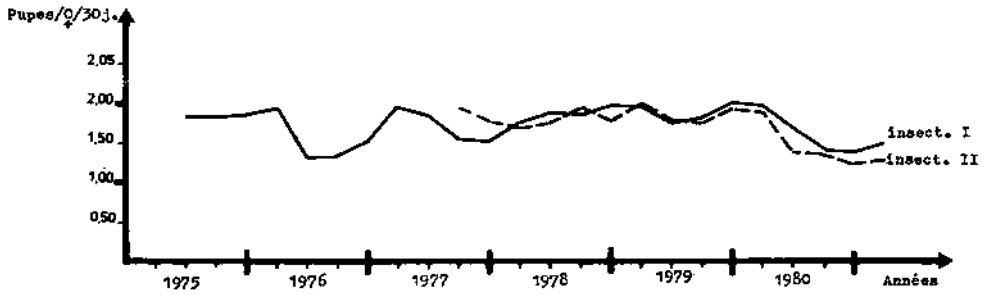

FIG.2. Evolution de la production de pupes par femelle par 30 jours.

2.2. **Productivité des femelles** (fig.2 et tab.I)

Elle est représentée par le nombre de pupes produites par femelle par période de 30 jours. Elle est calculée à partir de l'effectif moyen, jeunes femelles non reproductrices comprises.

La figure 2 montre que cette productivité a oscillé entre 1,5 et 2 en général, la moyenne se situant à 1,74 pour l'insectarium I et à 1,72 pour l'insectarium II.

Les chutes de productivité sont le fait, en premier lieu, de l'ingestion de sang contenant des antibiotiques [3, 4], en second lieu des perturbations climatiques (saturation d'eau, irrégularités thermiques) et des intoxications insecticides.

On peut dire qu'au dessus de 1,7 pupe par femelle sur 30 jours, l'élevage se porte bien; des valeurs inférieures indiquent un fonctionnement défectueux.

2.3. **Pourcentage d'éclosion** (tab.I)

Il représente le nombre de femelles et de mâles éclos pour 100 pupes produites. En fait, le taux d'éclosion dépend, d'une part, de la qualité de la pupe produite et, d'autre part, des qualités thermo-hygrométriques du lieu où se déroule la pupaison. Les chutes du pourcentage d'éclosion se rencontrent, en effet, soit lorsque la productivité des femelles est perturbée, soit lorsque le stockage des pupes est défectueux (humidité insuffisante ou trop grande, renouvellement d'air trop réduit, entassement des pupes, confinement dans des récipients trop profonds).

Il se situe en moyenne à 85,91% dans l'insectarium I et à 85,45% dans l'insectarium II. Son seuil critique est donc d'environ 85%.

2.4. **Mortalité des femelles** (fig.3 et tab.I)

Elle s'exprime par le nombre total de femelles mortes quotidiennement par rapport au nombre de femelles vivantes (mortalité journalière totale). Elle a été

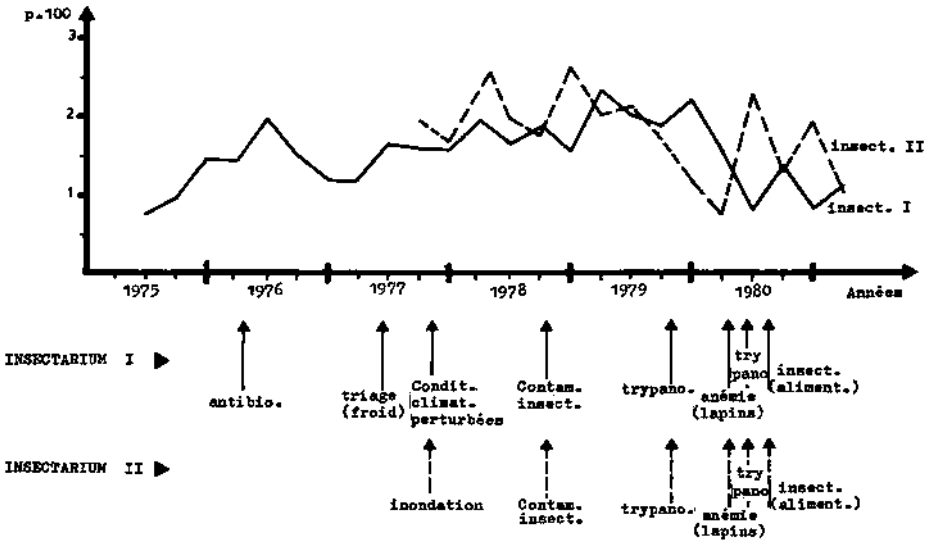

FIG.3. Evolution de la mortalité journalière totale des femelles.

en moyenne de 1,62% sur 6 ans dans l'insectarium I et de 1,85% sur 3 ans dans l'insectarium II, ce qui doit être considéré comme trop élevé dans les deux cas, car le taux de mortalité doit demeurer égal ou inférieur à 1,5%.

Cette mortalité globale concerne trois catégories de femelles:

– *les femelles mortes à l'éclosion* (femelles mortes/femelles écloses); le pourcentage est acceptable quand il demeure inférieur à 5%. Cette valeur a été dépassée lors des intoxications par insecticides ou lors de mauvaises conditions climatiques au niveau du lieu de stockage des pupes (trop forte ou trop faible humidité), en particulier dans l'insectarium I (moyenne: 5,69%). Elle est par contre bonne dans l'insectarium II (moyenne: 2,25%).

– *les femelles mortes entre le tri à l'éclosion et le tri après l'accouplement* (femelles mortes/femelles écloses – femelles mortes à l'éclosion); on remarque que, pour cette catégorie de glossines, la mortalité est beaucoup plus élevée dans l'insectarium II (moyenne: 12,33%) que dans l'insectarium I (moyenne: 9,10%) et ceci est très sensible en 1978 et 1979, période où le cobaye a été utilisé dans l'insectarium II. Son caractère remuant pourrait en être la cause (repas interrompu, traumatismes de l'appareil piqueur de la glossine). Le seuil critique est de 10%.

– *les femelles mortes après accouplement* (femelles mortes en 30 j. après accouplement/femelles vivantes pendant ces 30 jours); la mortalité est acceptable lorsqu'elle est inférieure à 40%, ce qui est le cas dans l'insectarium I (moyenne: 38,45%). Elle est beaucoup plus élevée dans la colonie nourrie sur cobayes où elle a atteint pour cette période 49,12% en moyenne.

TABLEAU II. POIDS DES PUPES DANS LES 2 INSECTARIUMS

	Insectarium I (sur lapins)	Insectarium II (sur cobayes)
1977	25,82 mg ± 0,44	26,01 mg ± 0,30
1978	26,12 mg ± 0,34	29,04 mg ± 0,30

2.5. Poids des pupes

Il apparaît que les glossines nourries sur cobayes donnent des pupes sensiblement plus lourdes que celles nourries sur lapins (tableau II).

3. DISCUSSION

D'un façon générale, il ressort que les caractéristiques biologiques de *G.p. gambiensis* élevée en Afrique sont au moins aussi bonnes que celles de la même espèce élevée en Europe et ceci malgré les difficultés beaucoup plus grandes de maintien de conditions thermohygrométriques stables en région tropicale (climat, fourniture aléatoire d'eau et d'électricité [5, 6]).

Il est donc indispensable d'être autonome en eau (forage ou château d'eau) et en électricité (groupes électrogènes puissants).

Par mesure de sécurité, l'élevage des glossines sera divisé en unités. Ces insectariums seront de taille moyenne, le plafond étant constitué d'une dalle en ciment (le bois est sujet aux moisissures et risque d'être toxique). L'installation en dehors des villes est très souhaitable (traitements insecticides urbains).

Dans ces insectariums, l'association de climatiseurs individuels et d'atomiseurs d'eau est fiable, robuste et facile à dépanner.

L'expérience nous incline à préférer le lapin au cobaye en zone tropicale humide

– *pour des raisons de rendement des colonies d'insectes:* taux de croissance supérieur, mortalité des jeunes imagos plus basse [6];

– *pour des raisons de commodité:* le lapin est calme, propre, facile à manipuler [6];

– *pour des raisons zootechniques et économiques* [7]: à prix d'entretien égal, le lapin a un taux de reproduction six fois plus élevé et permet malgré une mortalité deux fois plus forte que celle du cobaye d'avoir en fin d'année deux fois plus d'animaux vivants (tableau III), donc d'accroître plus rapidement les effectifs de glossines. Les races importées sont en effet fragiles et il est préférable d'avoir un élevage local autonome.

TABLEAU III. COMPARAISON ZOOTECHNIQUE DU LAPIN ET DU COBAYE A BOBO-DIOULASSO

	Lapin	Cobaye
Poids moyen	2923,20 g ± 110	568,80 g ± 49
Nombre animaux nourriciers pour alimenter 1000 glossines	1 X 6 d = 6 lapins	3,6 X 6 d = 22 cobayes
Coût d'1 lapin/1 cobaye	3,5	1
Nombre de petits nés par femelle et par an	24	4
Nombre de portées par femelle et par an	3,95	2,0
Nombre d'animaux prêts au travail par femelle et par an	12,7	2,6
Gain d'animaux par femelle et par an	2,9	1,6

4. CONCLUSION

L'expérience réalisée à Bobo-Dioulasso depuis six ans montre les possibilités et les limites d'un élevage de glossines en zone tropicale en cas d'utilisation du lapin ou du cobaye comme hôte nourricier. Celui-ci a permis cependant de produire 3 643 968 pupes et de lâcher près de 900 000 mâles irradiés.

L'ensemble des données recueillies indiquent que ces deux animaux conviennent bien à l'élevage de *G.p. gambiensis* avec toutefois des performances en faveur du lapin.

Leur élevage est cependant difficile et coûteux en zone tropicale et constitue un facteur limitant pour l'emploi de la technique du mâle stérile à plus vaste échelle.

Aussi des efforts de recherche sont-ils faits au CRTA pour la mise au point de l'alimentation sur membrane artificielle des glossines dont les premiers résultats très encourageants sont présentés au cours de ce colloque.

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PROTEIN ABSORPTION IN TSETSE FLIES, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae)

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Abstract

PROTEIN ABSORPTION IN TSETSE FLIES, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae).

Tsetse flies are able to absorb meal proteins undigested from the midgut into the haemolymph. Absorption of proteins is limited by the permeability of the peritrophic membrane. FITC-conjugated dextrans up to but not greater than a molecular weight of 45000 were shown to pass through the peritrophic membrane. A comparison of the protein pattern of blood serum and haemolymph of tsetse flies shows a congruency for albumin. With the help of immunological techniques it was possible to prove that the albumin fraction in the haemolymph in terms of immunological characteristics depends on the type of blood the flies are fed on. Albumin absorbed from the blood meal was found to be incorporated in various fly tissues, e.g. the fat body and ovary. It was shown further that albumin plays a role in lipid transfer from the fat body to the milk glands in female tsetse flies. These results explain a shortfall of lipid transfer in flies fed on an albumin-deficient diet. It may be that the quality of blood in respect to tsetse fly breeding is directly related to its albumin contents.

The idea that undigested meal proteins are absorbed from the gut lumen and directly incorporated into various tissues or organs sounds rather strange. In vertebrates the immune system does not allow protein absorption but also in lower animals it will not be the rule. There are, however, indications in the literature that certain insects are able to absorb proteins undigested from the gut. Meal proteins were shown to have entered midgut cells in *Ephestia kühniella* [1], haemolymph and other tissues of *Rhodnius prolixus* [2] and *Hypoderma bovis* [3] without being broken down by digestive enzymes. It has been shown also that immunoglobulins, e.g. antibodies produced against certain tissues of *Sarcophaga falculata* and administered orally to the fly pass through the gut wall [4] and react with the specific antigen [5]. The same technique has been applied to eliminate intracellular symbionts of the midgut mycetome of *Glossina m. morsitans* [6, 7].

Experiments have been conducted to determine how far serum proteins are protected from digestive enzymes, and are then absorbed and transported into the

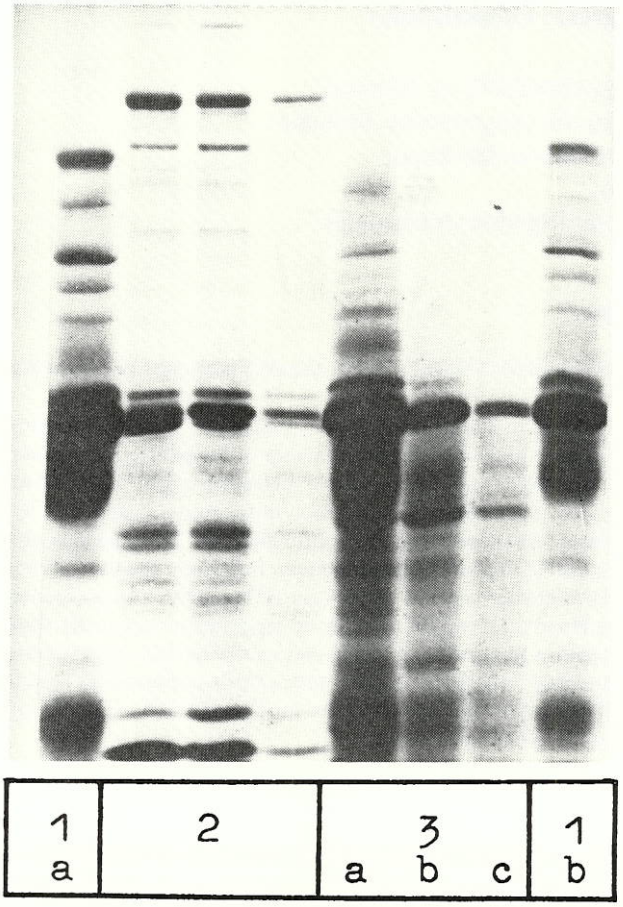

FIG. 1. SDS-Polyacrylamide gel electrophoresis.

1: bovine serum
1a: diluted 1:400
1b: diluted 1:800
2: haemolymph of tsetse flies
3: midgut contents
3a: 3 hours after feeding
3b: 12 hours after feeding
3c: 24 hours after feeding

haemolymph of tsetse flies. Absorption of proteins first of all is limited by the permeability of the peritrophic membrane. In comparison with other diptera, tsetse flies have a relatively thin peritrophic membrane. It was shown with the help of FITC-conjugated dextrans that the peritrophic membrane of tsetse flies allows the passage of molecules up to, but not greater than, a molecular weight of approximately 45 000. From these experiments it can be concluded that complete immunoglobulin, e.g. IgG (mol. wt. 150 000) may not pass through the peritrophic membrane. A passage through the membrane may be assumed, however, for fragments of globulins and possibly for albumin.

In fact, a comparison of the protein pattern of the blood serum and haemolymph of tsetse flies, when separated by cellulose acetate electrophoresis, reveals a striking congruency for albumin but no other fraction. A much better separation of proteins is achieved with SDS-polyacrylamide gel electrophoresis (Fig. 1). Here more than 20 different protein fractions are to be seen. These are compared with serum as well as the gut contents of flies 3, 12 and 24 hours after ingestion of a blood meal. Obviously the number of protein fractions is reduced during the process of digestion. At least one fraction, which corresponds to albumin in the blood serum, seems to be absorbed into the haemolymph.

To prove whether this and possibly other protein fractions are really identical with serum proteins, immunoelectrophoresis and immunodiffusion tests (Ouchterlony) were conducted [8]. With the help of these techniques it was possible to prove that the albumin-like fraction in the haemolymph of tsetse flies is identical with serum albumin, and in terms of immunological characteristics depends on the kind of blood the flies are fed on. The amount of albumin absorbed from the gut into the haemolymph was determined using quantitative immunoelectrophoresis after Laurell [9]. A linear correlation was found between absorption of albumin and the size of blood meal.

The occurrence of albumin in the haemolymph of tsetse flies is of particular interest in view of the findings of Langley and co-workers [10] and Takken [11], who were able to show that the presence of albumin in the diet is of greatest importance for tsetse fly reproduction.

Generally the absorption of proteins which do not have to be broken down to amino acids and to be synthesized again after absorption, means a saving of energy, a fact which may be of high importance for an insect which depends totally on an energy-poor diet such as blood. Experiments in progress have shown that albumin absorbed into the haemolymph is transferred further into other organs, e.g. into the fat body and ovary. It is interesting to note that Takken [12] observed atrophy of the ovaries when tsetse flies are fed on an albumin-deficient diet.

Alternatively or additionally, albumin may have a vehicle function in the haemolymph of tsetse flies comparable to its function in vertebrate blood. In particular we pursued the assumption that albumin is involved in lipid transfer.

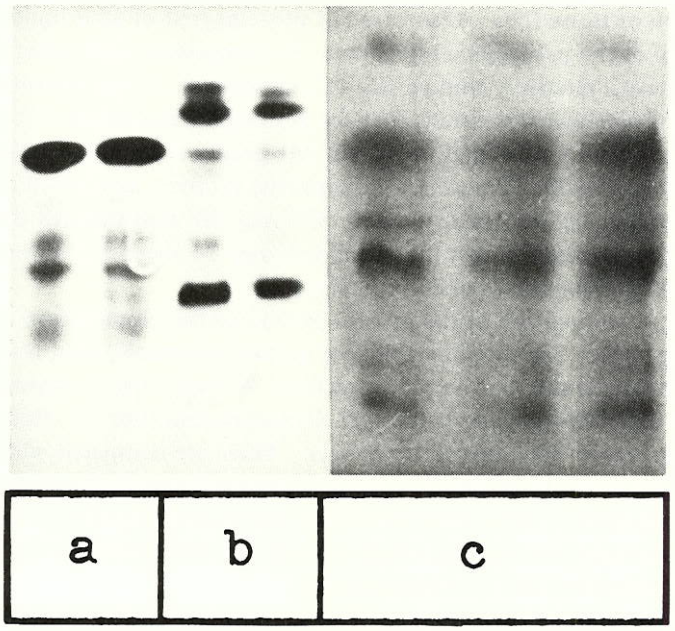

FIG.2. Distribution of radioactivity in the haemolymph of G. m. morsitans *after a single injection of* [U-^{14}C] *leucine in early pregnancy. Electropherograms of blood serum (a), haemolymph of female flies on day 6 of the pregnancy cycle (b), and autoradiographies of haemolymph electropherograms (c).*

If radioactive [U-^{14}C] leucine is injected into females during the early stage of pregnancy, e.g. the second day, about 70% of the recovered radioactivity is found to be incorporated in the lipid fraction of the fat body. During the late pregnancy cycle a massive transfer of lipids can be observed from the fat body to the milk glands which provide the growing larva with a nutritive secretion [13].

In order to investigate the role of albumin in the transfer of lipids from the fat body to the milk glands during the reproductive cycle, experiments were undertaken in which the same technique of injecting [U-^{14}C] leucine was used as described by Langley and Bursell [13]. Haemolymph was collected from the flies and separated with the help of a cellulose acetate electrophoresis. The electropherogram was then exposed to an X-ray film (Fig. 2). As can be seen, the main amounts of radioactivity are associated with the two protein fractions, one of which corresponds to the albumin fraction.

The radioactivity of this fraction was determined quantitatively daily during the pregnancy cycle with a liquid scintillation counter after the fraction had been cut out from the cellulose acetate foil (Fig. 3). There is a sharp increase in the

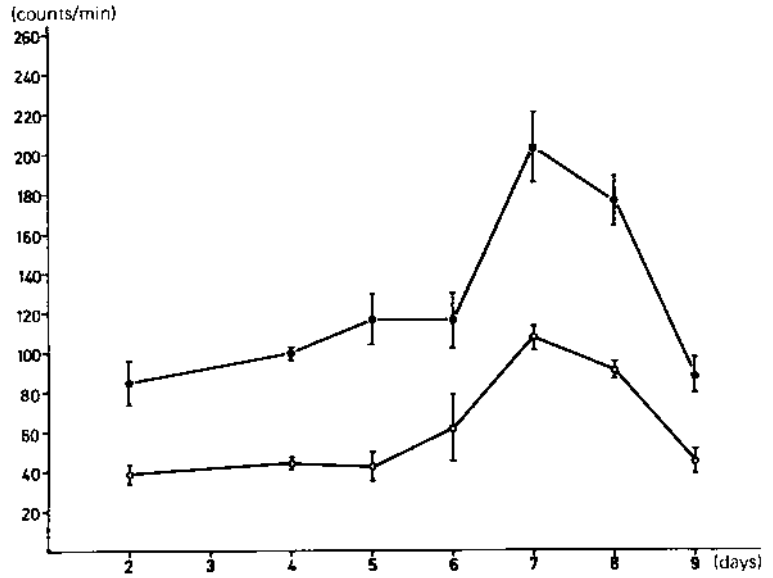

FIG.3. Distribution of radioactivity in the haemolymph of G. m. morsitans *during the 2nd pregnancy cycle after a single injection of* [U-^{14}C] *leucine on day 2 of the cycle.*
●—● *total recovered radioactivity*
○—○ *radioactivity associated with the albumin fraction*

radioactivity in the late pregnancy cycle, a result which is quite in agreement with the findings of Langley and Bursell [13]. These results clearly provide evidence that albumin plays an important role in the lipid transfer of female tsetse flies. Certainly albumin is not the only lipid carrier, but about 45% of the total recovered radioactivity was found in the albumin fraction.

It is well known that bovine blood as a diet for tsetse flies leads to production of undersized offspring in comparison with porcine blood [14]. This seems not to be influenced by the sizes of blood meals or the rate of digestion. Females of *G. m. morsitans* even synthesize identical amounts of lipid in their fat body and also at the same rate regardless of whether they are fed on porcine or bovine blood [15]. The deposition of lipids in *G. p. palpalis* is even higher with bovine than with porcine blood (Feldmann, unpublished). However, there is an obvious shortfall of transfer of lipids of at least 1 mg from the fat body to the growing larva in the uterus when the flies are fed on bovine blood [15]. This fact is reflected also by the cycle of milk gland growth. The milk gland tubules of flies fed on bovine blood are unable to achieve the same increase in size as those of flies fed on porcine blood. In the light of these results it is interesting to know that porcine blood

TABLE I. ALBUMIN CONTENTS OF DIFFERENT TYPES OF BLOOD USED FOR BREEDING *G. morsitans* AND PUPARIAL WEIGHTS ACHIEVED

Type of blood (and feeding regime)	Albumin contents (g/100 ml serum)	*G. morsitans* puparial weight (mg)	Refs
Rabbit (live)	4.45	30–32	[17]
Pig (membrane)	3.2	30.2	[14]
Goat (live)	3.17	29.9	[14]
Cow (membrane)	2.93	26.1	[14]
Horse (membrane)	2.89	28[a]	[18]
Sheep (membrane)	2.68	23.5	[19]

[a] Selection of pupae over 24 mg.

has an albumin content of 3.2% whereas bovine blood has only 2.93%. In Table I different mammals, the blood of which has been used for breeding *G. morsitans*, are listed in the sequence of albumin contents [16]. The second column shows the puparial weights achieved. It may be that the quality of blood in tsetse fly breeding is directly related to its albumin contents.

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CHANGES IN THE EGG OF THE TSETSE FLY, *Glossina palpalis palpalis* (Diptera: Glossinidae), AFTER FERTILIZATION BY SPERM OF GAMMA-IRRADIATED MALES

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Abstract

CHANGES IN THE EGG OF THE TSETSE FLY, *Glossina palpalis palpalis* (Diptera; Glossinidae), AFTER FERTILIZATION BY SPERM OF GAMMA-IRRADIATED MALES.

This study was undertaken to establish the pattern of normal embryonic development as a basis for comparing aberrant development that may occur when females are mated to radio-sterilized males. Seven- to ten-day-old males of *G. p. palpalis* (Nigerian origin) were irradiated in air with 12–13 krad gamma rays from a ^{60}Co source. Three hours after treatment they were allowed to mate with two-day-old normal females. Reproductive organs from females mated with treated males and with normal males were removed at regular intervals (starting on the eighth day following emergence) and examined by histological techniques. Extruded eggs were also examined. Development of most eggs fertilized by sperm of irradiated males was inhibited in cleavage and lytic processes took place on the following days. In eggs that ceased to develop and were later expelled at irregular intervals, the chorion was separated from the choriothete prior to extrusion. The occasional findings that embryogenesis was not interrupted until advanced stages or that normal embryonic and larval development was completed, indicate that treatment doses of 12–13 krad do not entirely damage the genetic material of the nucleus of sperm, or that repair is possible to some extent. Cyclic changes of the choriothete during pregnancy, its secretion and changes observed during this study, imply that, besides facilitating nidation of the egg in the uterus, the choriothete might function in folding of the chorion on the ventral floor of the uterus.

Seven-to ten-day old males of *Glossine palpalis palpalis* (Nigerian origin) were irradiated in air with 12–13 krad gamma rays from a ^{60}Co source.[1] Three hours after treatment they were allowed to mate with two-day-old normal females. Reproductive organs from females mated with treated males and with normal males were removed at regular intervals (starting on the eighth day following emergence) and examined by histological techniques. Extruded eggs were also examined.

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Cyclic changes of the choriothete during pregnancy, its secretion and changes observed during this study, imply that, besides facilitating nidation of the egg in the uterus, the choriothete might function in folding of the chorion on the ventral floor of the uterus.

The tsetse fly, *Glossina palpalis palpalis* (Rob.-Desv.), is widespread in west Africa and acts as an important vector of human and animal trypanosomiasis. As with the other economically important tsetse, the west African species *G. p. gambiensis* and *G. tachinoides,* and the east African species *G.m. morsitans* and *G. pallidipes,* the reproductive biology and control of fecundity of *G.p. palpalis* have been and still are the subject of extensive studies.

Because of particular features of the insect's biology it is difficult to interfere with its reproductive cycle. Adaptive mechanisms that enable reduction of the number of offspring on one hand, ensure on the other that mortality is minimal from egg to adulthood. This is due to adenotrophic viviparity [1–3] whereby the entire embryogenesis and larval development take place in a uterus. For ovulation to occur the tsetse female must have a mature chorionated egg in the ovary, and must have copulated [4]. Larval stages in utero are nurtured to maturity in the maternal organism by secretions from a specialized accessory gland. Pupariation follows soon after deposition of the third instar larva. The ovaries are much reduced in number; the left and right ovary each contain two ovarioles which show alternate sequential development. The accuracy with which the cyclical events in the reproductive system are regulated forms the basis for physiological age determination [3, 5].

Any factors that affect reproduction of insects have immense practical consequences for control strategies. For example, a promising approach to solving

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[1] 1 rad = 1.00×10^{-2} Gy.

the tsetse-trypanosomiasis problem in Africa is the implementation of vector control campaigns that are based on the use of radiosterilized males [6, 7].

Changes in the development of eggs obtained from female *G. p. palpalis* after their mating with gamma-irradiated males are described below. A concise analysis of normal embryonic development was used as a basis for observations of changes in development of eggs isolated from the uterus or eggs expelled by females mated to irradiated males.

MATERIAL AND METHODS

Experiments were set up using adult flies from a colony of *G. p. palpalis* (Nigerian origin) fed in vivo on guinea pigs. Details of the rearing procedures are reported elsewhere in these Proceedings [8].

Two batches of 100 females were sampled randomly for studying follicle development, egg maturation and timing of ovulation during the first reproductive cycle under standard holding conditions (24.5°C, 85% R.H. and 6 feeding days/week). The two groups of females, which emerged on the same day from randomly sampled puparia (weight range: 24 mg to 34 mg initial weight), were mated to untreated males. Oocyte development and uterine content in those females were checked at ages 7 and 14 days respectively.

Experimental males were irradiated in air when seven- to ten-day-old with 12–13 krad gamma rays from a ^{60}Co source, and allowed to mate with two-day-old stock colony females three hours later. Females mated to treated males and females mated to normal untreated males were immobilized at regular intervals (starting on the eighth day following emergence) and dissected. Female reproductive organs were isolated from the abdomen and examined under the binocular microscope. After fixation in Bouin or Carnoy, the organs were transferred to 80% alcohol and processed through the usual histological techniques. Paraplast sections 4–6 μm thick were stained with Meyer's haematoxyline. Extruded eggs collected from the larviposition trays containing 0.9% NaCl were similarly processed.

Observations on follicle development, egg maturation and ovulation in control females, together with radiation treatments and dissection of the experimental material, were carried out in the IAEA Seibersdorf laboratory. Histological processing and embryological evaluation was done in Prague.

RESULTS

Observations on follicle development

Figures 1 (7-day-old females) and 2 (14-day-old females) indicate that females taken at random and kept under a standard feeding regimen and optimal environmental conditions, displayed considerable variation in their pattern of follicle

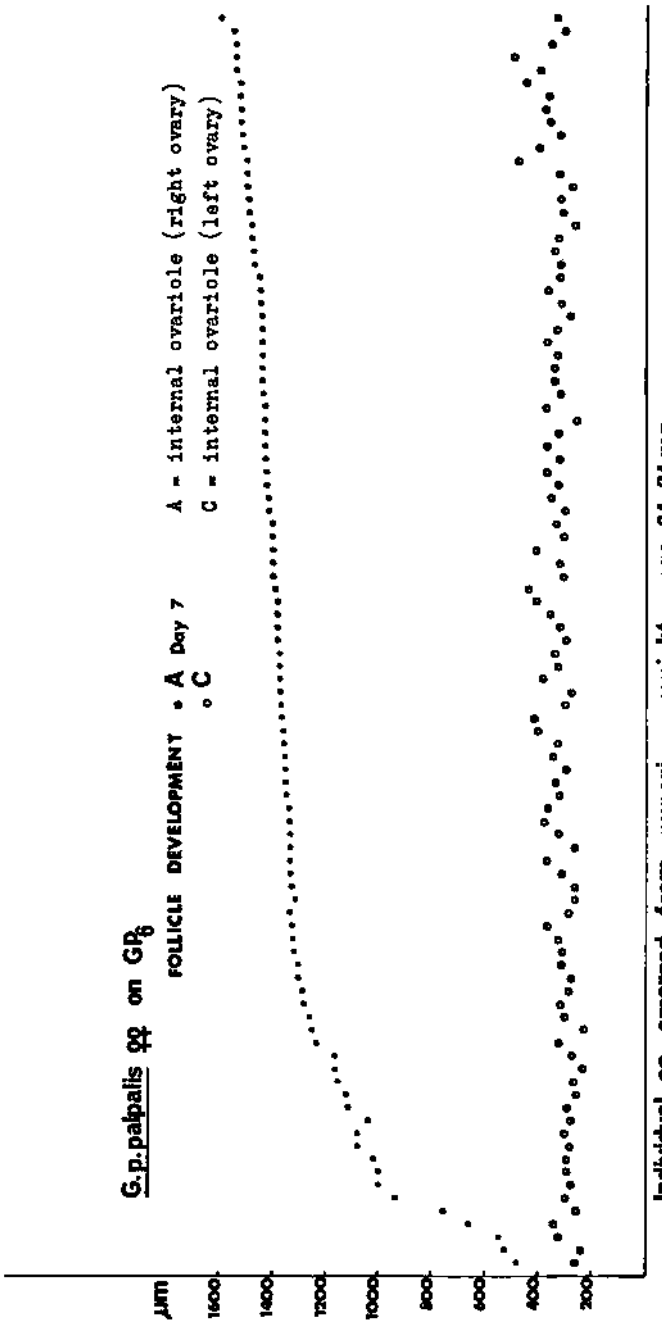

FIG. 1. Follicle development in seven-day-old females.

growth and timing of their first ovulation (Fig. 3). Vitellogenesis in the first functional follicle (internal ovariole A of the right ovary) begins near the end of the pupal stage (about five days before emergence) and continues under the influence of blood ingested during the first week of adult life. The observed variation in actual follicle size (on days 7 and 14) and uterine content (on day 14) for females that emerged from puparia weighing between 24–34 mg, emphasizes the importance of initial puparial weight and embryonic reserves in the newly emerged fly. Thus, deficiences in initial puparial weight of 5 to 10 mg may account for a delay in first ovulation of at least two to four days. This was considered when the chronology of the early stages of embryogenesis was studied in control females and females mated to irradiated males.

Embryonic development in control females

First ovulation occurred on the eighth day after emergence. At the time the mature egg was about 1.45 mm long and attached at the anterior ventral part to the choriothete. Cleavage division was distinct but, because development was not synchronous in all flies, the numbers of cleavage nuclei differed in individual eggs. In the most advanced stage the cleavage nuclei had migrated to the periphery of the egg. During the ninth day, embryo development reached the blastoderm stage with a cap of pole cells or even the more advanced stage of gastrulation (Fig. 4). Generally, ovulation was completed in most females by the tenth day, and the embryos had attained the segmentation stage and various stages of organogenesis.

During day 11, the majority of eggs contained advanced embryonic stages that were close to hatching into first instar larva. The chorion began to crease in the area of the choriothete. Before this folding the choriothete shows villous structure (Fig. 5). Also at this stage, the larval gut already contained milk gland secretion.

On day 14, most of the in utero larvae were in the second instar stage and some had already reached the early third. The uterus was distended by the longitudinal growth of larva, the choriothete was flattened and chorion and larval exuviae were folded on the ventral side until parturition (Fig. 6). The first larviposition may occur on day 16 following emergence, but for most females it occurs between days 18 and 21. At this stage, the next follicle in sequence (ovariole C in right ovary) has almost reached maturity (follicle size 1.4 to 1.5 mm).

Embryonic development in females mated to irradiated males

As in the control females, ovulation in females mated to irradiated males began on the eighth day following emergence, and the choriothete in the ventral wall of the uterus was of a columnar structure. Cleavage division was apparent in the eggs (Figs 7 and 8), but the periblast was found also (Fig. 9).

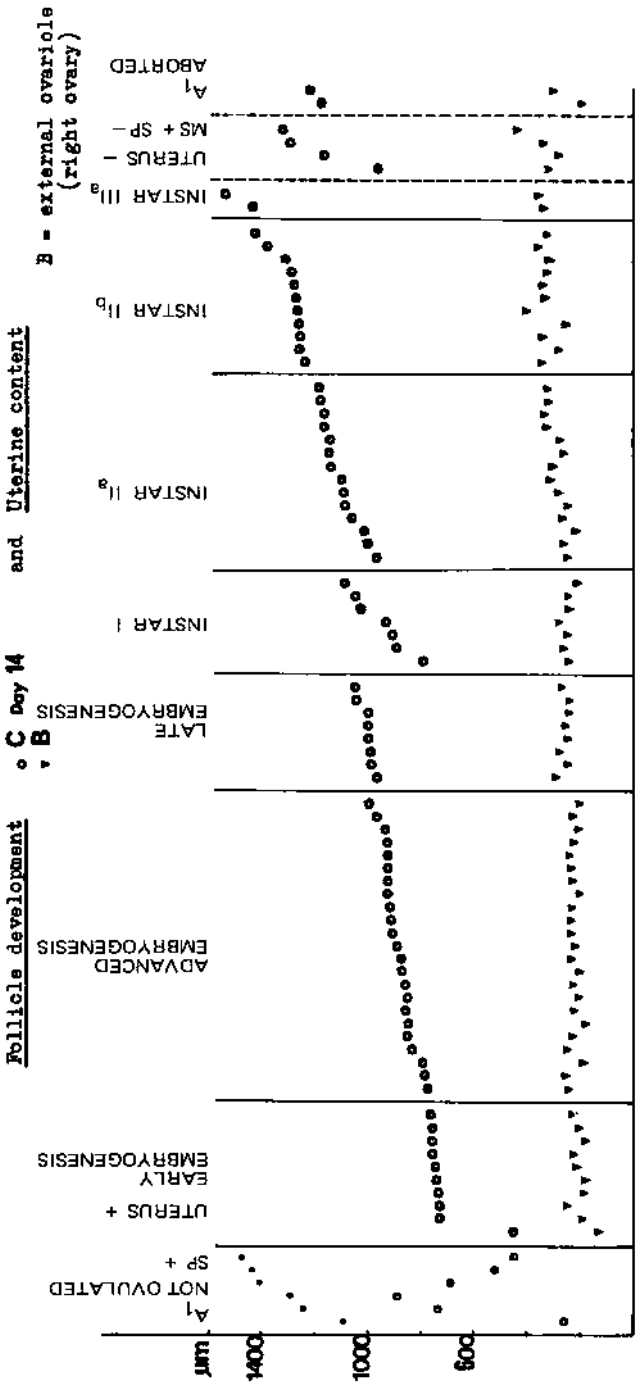

FIG. 2. Follicle development in fourteen-day-old females.

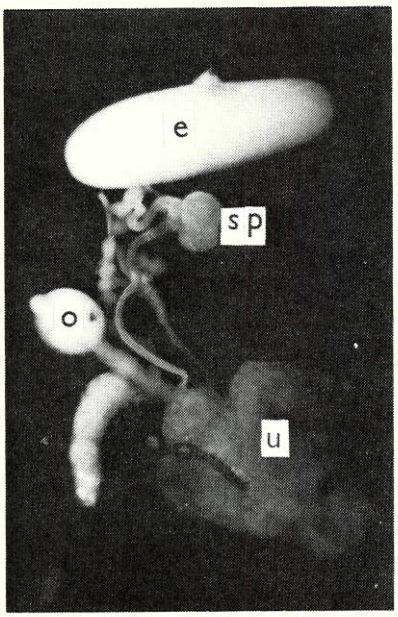

FIG.3. Female reproductive system dissected before ovulation: e = egg; o = ovariole; u = uterus; sp = spermatheca.

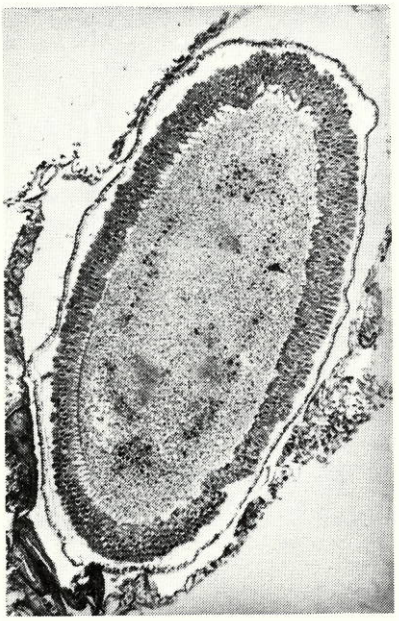

FIG.4. Gastrulation.

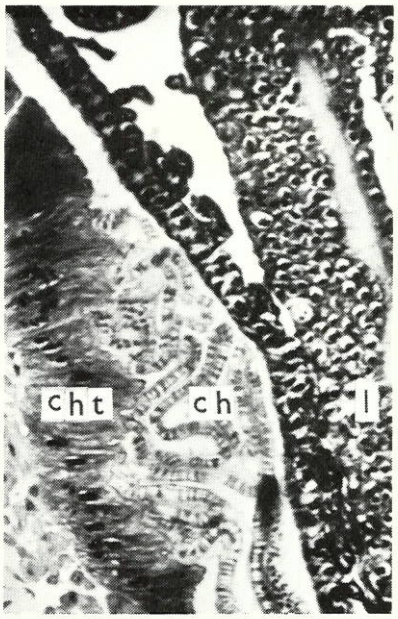

FIG. 5. Detail of choriothete showing villous structure: cht = choriothete; ch = chorion; l = larva.

FIG. 6. Larva in the uterus, choriothete and creased chorion: ch = chorion; l = larva.

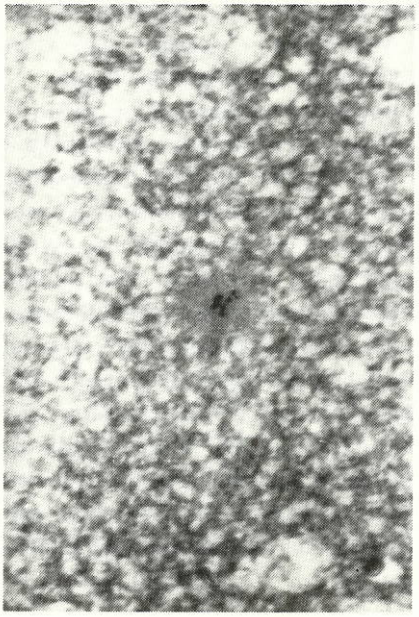

FIG. 7. Cleavage nucleus.

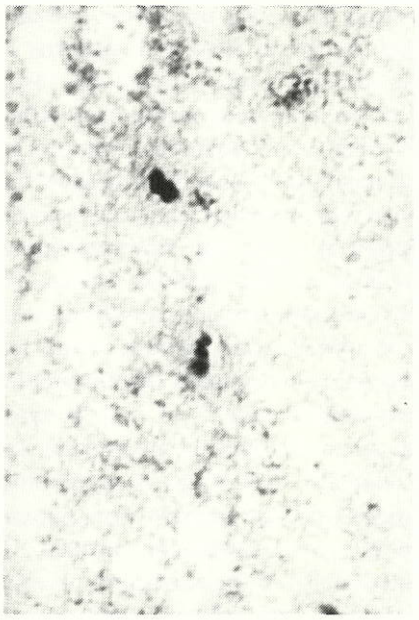

FIG. 8. Development inhibited in cleavage division.

By day 9 most females had their first ovulation, but development of the egg had largely ceased at cleavage division; cleavage nuclei were of various sizes. Normal development to gastrulation was extremely rare.

After day 10 lytic processes were observed in about 95% of all ovulated eggs; in most cases caryolysis had apparently advanced so far that nucleic material could not be identified. The presence of structures resembling the cytoplasmic halo of cleavage nuclei indicated that development had been blocked at cleavage or at the migration of cleavage nuclei into superficial cytoplasm. Chromosomal material was rarely distinguishable several days after inhibition of cleavage.

Eggs that had ceased developing were expelled between days 14 and 20 following emergence, i.e. between five and eight days following ovulation. In those females which expelled their eggs (88% of all experimental females) it was observed that the egg, a few days before extrusion, was no longer closely associated with the choriothete (Fig. 10). Moreover, the structures in extruded eggs indicated that development had been blocked at the stage of cleavage division. Both nuclei and yolk had disintegrated; the latter was very vacuolated. Only rarely was an egg expelled in which the embryo died at an advanced stage.

DISCUSSION

The results of this study show that irradiation of male *G. p. palpalis* with 12–13 krad in air induces changes in the genetic material of sperm that cause inhibited development in the early stages of most eggs fertilized by it. This effect is believed to be caused by the induction of dominant lethal mutations, and the lethal event is expressed when genetic material begins normal functions. The lethal mutations are frequently manifested by derangement of the first cleavage division and less often development is inhibited at advanced cleavage or at migration of cleavage nuclei to the periphery of the egg. Embryos rarely died at an advanced stage. These findings are in accordance with the effects of radiation on dipterous insects with monokinetic chromosomes as generalized by LaChance [9, 10].

A concise analysis of normal embryogenesis, which served as a basis for the study of changes in embryonic development when irradiated males were involved, confirmed previous embryological data [1, 11, 12]. It has been found that not all eggs sampled at one time are at the same developmental stage. Some of the differences are directly related to differences in initial follicle growth and pattern of egg maturation in females that had emerged from puparia of different weights. All eggs that ceased to develop were eventually expelled. Questions remain unanswered about the mechanism of egg extrusion: whether it is regulated neuro-humorally, a function of a physiological clock, an effect from the choriothete or because of toxic effects from the disintegrated egg.

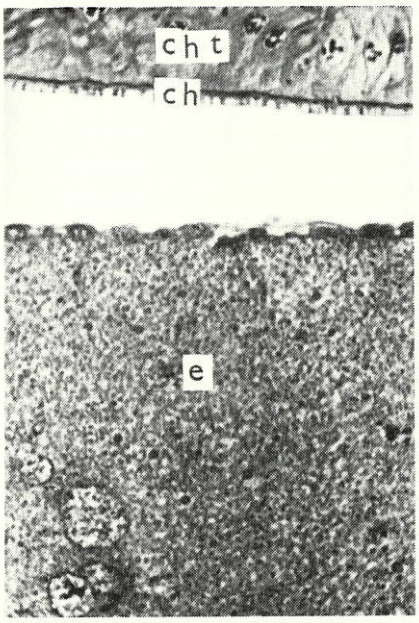

FIG.9. Periblast, chorion attached to the choriothete: cht = choriothete; ch = chorion; e = egg.

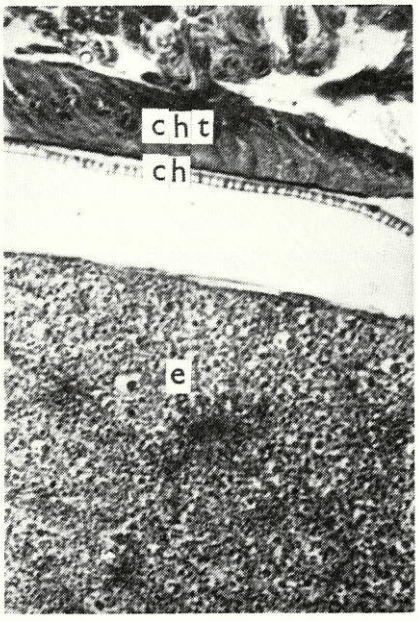

FIG.10. The beginning of extrusion, karyolysis after the blocking of embryogenesis in cleavage division; cht = choriothete; ch = chorion; e = egg.

Under optimal conditions (24.5°C; 85% R.H. and an adequate feeding regimen), the embryonic period in *G.p. palpalis* lasts 3.5 days for a total pregnancy period of 9.5 days. Starvation during pregnancy, severe ambient conditions [11, 13] and application of juvenile hormone or ecdysone [14] cause abortions. Stress conditions predominantly result in extrusion of immature and undersized larvae, and abortions in the egg stage are very infrequent. The situation is different for females mated to radiosterilized males as proven in our experiments which show that eggs were extruded on days 14 to 20 following emergence. However, the few cases in which the whole embryonic and larval development was normal clearly show either that doses of 12–13 krad do not entirely damage genetic material of the nucleus of sperm or that repair is possible to some extent.

When abortion occurs in females that were fertilized by fertile sperm the development and ovulation of the next egg follicle is accelerated [15, 16]. The same events happen as soon as embryonic development ceases in females that have been inseminated by irradiated males [17, 18]. In such cases, the uterus with the degenerating egg may offer enough space for ovulation of the next egg in sequence that, as a result, develops one to two days earlier than normal. Thus, in older females, eggs from two consecutive reproductive cycles might be found in utero. Moreover, prolonged retention of degenerating egg(s) in the uterus might cause ovary blockage [19].

The role played by the choriothete in females with incomplete embryogenesis still needs elucidation. There are many hypotheses on the function of this special structure in the ventral wall of the uterus that was first described by Jackson [20]. Hoffmann [2] has suggested that it lyses the chorion. The choriothete serves as a means of removal of the chorion [21] or both chorion and exuviae of the first and second larvae [22]. Roberts [23, 12] has stated that the choriothete is an organ that supports the developing embryo and larva, and that the chorion is punctured by the egg tooth at hatching. Cyclic changes of the choriothete during pregnancy [24], its secretion [23] and changes observed by us, imply that besides facilitating nidation of the egg in the uterus, this structure might function in the folding of the chorion on the ventral floor of the uterus. It can also play an important role in extrusion of non-developing eggs.

ACKNOWLEDGEMENTS

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STERILITY INDUCTION IN TSETSE

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Abstract

STERILITY INDUCTION IN TSETSE.

The first laboratory and field experiments on genetic control were with tsetse flies, and they made use of the sterility in crosses and hybrids between closely related species of the *Glossina morsitans* complex. Backcrosses indicate that there are two separate sterility mechanisms involved: (a) genetic incompatibility between a mother and the products of alien genes in the embryo or larva in the uterus; (b) inability of hybrid males to inseminate due to incompatibility of their X-chromosomes with an alien Y or autosomes. The two largest sterile male release programmes have been in Tanzania and Upper Volta, and have used irradiation at the pupal or adult stage, respectively, for the production of sterility. Male tsetse are remarkably resistant to radiosterilization and, with the doses required to induce dominant lethals in more than 95% of sperms, premature senescence and lethargic behaviour of the males tends to result. With *G. morsitans* irradiated at the puparial stage these effects can be alleviated by the use of a nitrogen atmosphere during irradiation. If the puparia are then transferred to air at 11°C for transport to the release site, immediate emergence occurs on re-warming after arrival. This advantageous procedure was used for the releases in Tanzania. In addition to dominant lethals, irradiation also produces chromosome translocations which cause inherited partial sterility. A homozygous translocation line was selected but this example did not have sufficient fitness to be used in a mass rearing programme. Chemosterilants can be applied by pupal dipping, adult contact with deposits or in aerosols. Studies are now in progress on their use in association with odour-baited traps or pheromone-baited decoys as a means of sterilizing the wild population and thus avoiding the costly and difficult process of mass rearing tsetse.

Induction of sterility in tsetse flies has been studied intermittently over the last 45 years with the Sterile Insect Release Method in mind.

STERILITY FROM HYBRIDIZATION

The earliest studies were those of Potts [1] and Vanderplank [2–4] on the sterility which occurs when closely related species or subspecies of the *Glossina morsitans* complex are crossed. There are two distinct sterility or partial sterility mechanisms involved: (a) partial sterility in the crosses and the female hybrids, and (b) sterility in the male hybrids [5]. The two reciprocal crosses between a pair of species or subspecies generally differ in their level of sterility, and in some cases one of the reciprocal crosses produces very few puparia which show a poor hatch rate and a poor survival of any emerged adults. The difference between the reciprocal crosses might suggest that this is another example of uni-directional cytoplasmic incompatibility. However, this has been shown not to be so, because when the female F_1 hybrids are backcrossed in various ways there is partial sterility in all cases (Fig.1). If the sterility were due to maternally inherited cytoplasmic factors, the female F_1 and the backcross progeny shown in Fig.1 would have the crossing properties of *G. m. morsitans*, and would be fully fertile when crossed to males of this subspecies. The results are consistent with the hypothesis that, depending on their own genetic composition, female flies react adversely to products of genes alien to themselves in the embryo or larva in the uterus, the reaction being more violent between *G. m. centralis* mothers and *G. m. morsitans* progeny than in the reciprocal case. On this hypothesis, the last cross in Fig.1, for example, would be between a mother which was predominantly *centralis* in its genetic composition and progeny which were more than 50% *morsitans*. It is to be expected, therefore, that the fertility would have been low but not quite as low as the *centralis* female × *morsitans* male cross. Similar results were obtained in combinations where the level of fertility in the original crosses was considerably lower. Such a mother-embryo incompatibility, with a difference between reciprocal crosses, is formally analogous to Rhesus blood group incompatibility in humans.

The male F_1 hybrids from the crosses in Fig.1 are completely sterile because the sperm is unable to travel from the spermatophore to the spermathecae [5]. From backcrosses there is a segregation of males with normal inseminating ability and those without it. The use of the X-chromosome marker *ocra* showed that a major factor in causing this male sterility is incompatibility between the X-chromosome of one subspecies and the Y or autosomes of the other. Figure 2 shows that there was sterility in nearly all those backcross males where the origin of the X-chromosome did not 'match' that of the Y or the majority of the autosomes. Conversely, where these did 'match' the proportion of fertile males was higher [6].

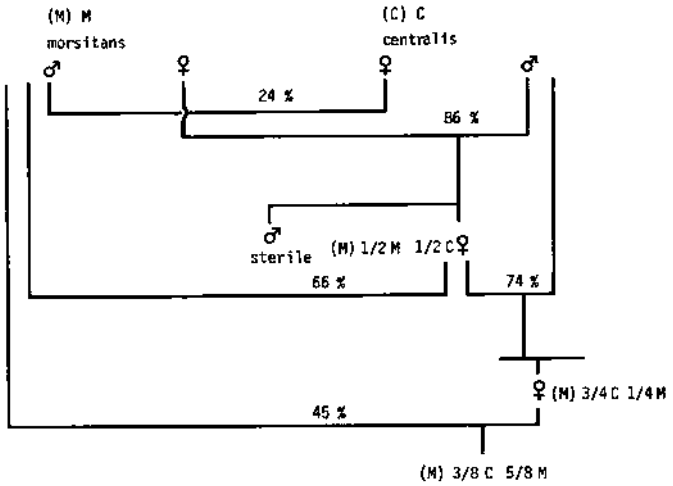

FIG.1. Crosses between G. morsitans morsitans *and* G. m. centralis. *The percentages indicate the fertility (production of puparia/ovulation cycle) relative to within-subspecies control matings. The genetic composition of each generation is indicated in terms of the contribution from the* morsitans *and* centralis *parents. Symbols in parentheses represent any maternally inherited factors, the other symbols with fractions refer to the proportions of autosomal genes from each parent. Data from Refs* [5] *and* [6].

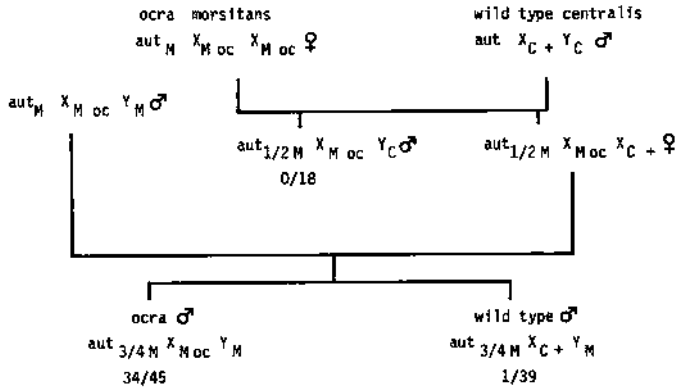

FIG.2. Cross and backcross with G. m. morsitans *(with the X-chromosome marker with* ocra*) and wild type* G. m. centralis. *The figures indicate number of males able to inseminate/total males tested. The genetic composition of each type is indicated with respect to autosomes (aut), X-chromosome and Y-chromosome; M and C represent chromosomes of* morsitans *or* centralis *origin; oc =* ocra *mutant, + = its wild type allele. Data are from Ref.* [6].

TABLE I. DATA OF JACKSON [7] ON PAIRING BETWEEN *G. swynnertoni* AND *G. morsitans centralis* FOLLOWING RELEASES OF PUPAE OF BOTH SPECIES IN A *swynnertoni* HABITAT

Data derive from identification of copulating couples

Estimated ratio of morsitans: swynnertoni *released = 5.24:1*

Male / Female	*swynnertoni*	*morsitans*	Total
swynnertoni	61	7	68
morsitans	299	41	340
Total	360	48	408

Heterogeneity $\chi_1^2 = 0.042$, not significant.

Note: The disproportionality between the total numbers of males and females of each species is thought to be because many of the released *morsitans* males had dispersed before becoming sexually potent whereas wild *swynnertoni* males were present and probably taking part in the mating.

The usual objection to the use of hybrid sterility for genetic control is that differences in the mate recognition systems of the species will prevent cross matching in the field. However, such barriers may not actually be a problem in the *G. morsitans* complex, where, in the mid-1940s Jackson [7] found random pairing in the field when *G. m. centralis* and *G. swynnertoni* males and females were released together (Table I). Following this work, Vanderplank [3] (and personal communication) released *G. m. centralis* puparia collected from their natural habitat into a drier area inhabited by *G. swynnertoni.* Hybrids appeared in the population for a few months, the *G. swynnertoni* population was suppressed and later the released *G. m. centralis* died out, apparently because of their intolerance of the dry conditions of the release area. Any form of genetic control not requiring artificial rearing is of great interest, but such a fortunate outcome could not be relied upon in other cases, and the danger of replacement of one population by another more harmful and viable vector should not be overlooked.

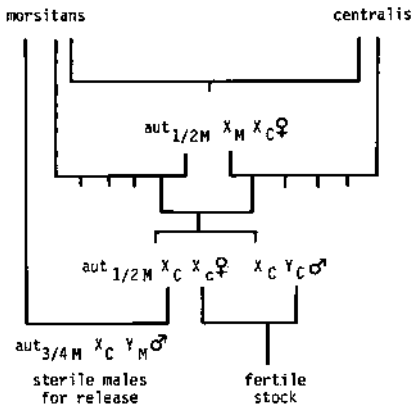

FIG.3. Proposed crossing scheme to produce a 'synthetic' fertile stock with its X- and Y-chromosome from G. m. centralis *but its autosomes partly from* G. m. morsitans. *Outcross of the synthetic stock to* G. m. morsitans *could produce sterile males for use against the latter species.*

This risk would be avoided if one only aimed to make use of the male hybrid sterility and, by analogy with the situation in *Anopheles gambiae* [8], with a suitable backcrossing programme one could probably synthesize a fertile strain which, on outcrossing to one of the pure species, would give males with their autosomes predominantly from that species but with a 'foreign' X-chromosome which would render them sterile (Fig.3). There would be no chance of an accidental population replacement if such males were released. Their possible advantages over radio-sterilization are that the cost of a radiation source and harmful somatic damage due to radiation would be avoided. However, no sperm is introduced into the spermathecae by such hybrid males, and the question arises as to whether their mates would be readily re-mated and rendered fertile by wild males. Limited tests in the laboratory [5] showed that sterile hybrid males had some ability to deter their mates from re-mating, and more recent studies on the decline of receptivity in mated *G. m. morsitans* females shows that this is caused by physical and chemical stimuli independent of sperm transfer [9]. Therefore hybrid males would presumably be able to bring about normal reductions in the receptivity of their mates.

The sisters of sterile hybrid males produced as proposed above would be expected to be almost fully fertile, and it would be essential to remove all females before release, otherwise the net effect of a release programme could be seriously reduced, or even rendered counter-productive by adding more breeding females to the population than are eliminated from it by the activities of the males. The required high degree of sex separation could only be achieved by delaying releases until after emergence.

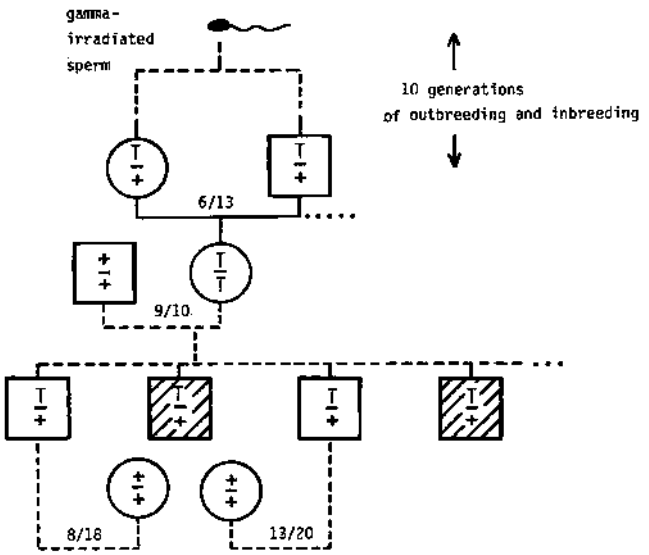

FIG. 4. Part of the pedigree of a strain of G. austeni *in which an autosomal translocation had been induced [17]. Squares = males, circles = females, +/+ = wild type, T/+ = translocation heterozygote, T/T = translocation homozygote, solid lines represent inbreeding, dashed lines represent outbreeding to known wild types, cross hatching represents individuals identified cytologically, figures represent no. puparia/no. ovulations. The T/T individual was nearly fully fertile and was identified by the fact that on outbreeding it produced all T/+ individuals which are semi-sterile and identifiable in the puparium by abnormal chromosome pairing.*

RADIATION STERILIZATION

The recent field trials in Tanzania and Upper Volta have both used gamma-radiation sterilization applied at the late puparial or early adult stages respectively. Tsetse females are sterilized by as little as 1 krad (= 10 Gy), so that any inadvertently released irradiated females could not reproduce. The dose required for the sterilization of tsetse females is less than that in other Diptera, e.g. in *Cochliomya hominovorax* where the dose of 7.5 krad used in the release programmes is dictated by the level required for sterility of the females which are released with the males without any attempt at sex separation. Male tsetse, however, are more resistant to radiation sterilization than any of the other Diptera so far tested [10]. With all species of tsetse that have been tested, doses in excess of 10 krad gamma rays are needed to ensure that more than 95% of sperm will carry dominant lethal mutations [11–16]. The greater susceptibility of female tsetse and greater resistance of male tsetse in comparison with other Diptera remains unexplained, but it serves to underline the fact that radiobiological effects cannot be explained

on a simple-minded 'bullet and target' theory, but on the contrary complex biochemical processes intervene between the arrival of a high-energy photon and the final biological effect.

At the large doses required to produce high rates of dominant lethality in male tsetse there is considerable induction of chromosome translocations and of somatic damage.

Translocations cause inherited partial sterility and they have been selected, replicated and observed cytologically in *G. austeni.* Figure 4 shows part of the pedigree of an autosomal translocation which can be inherited by either sex [17]. The only practicable way to rear tsetse translocations would be as viable and fertile homozygotes. Different homozygotes would be crossed to produce highly sterile multiple heterozygotes for release. Homozygotes for the translocation shown in Fig.4 were produced [17], but the viability and fertility of homozygotes for this particular translocation was insufficient to contemplate mass rearing.

The rate of occurrence of new translocations in the F_1 progeny of males irradiated with 6–15 krad is about 40% and the resultant partial sterility would contribute an appreciable 'bonus' to a release programme with irradiated males [18].

The most easily quantified somatic effect of irradiation is the induction of premature senescence, i.e. the life table (graph of percentage of survivors against age) bends downward earlier than usual [11, 19]. Marked releases of irradiated, reared males and unirradiated wild males of *G. palpalis* were made in Upper Volta by Clair and co-workers [20]. Subsequent trapping showed a considerably shorter lifespan in the former type of male than the latter. The lifespan in the field of irradiated males has more recently been found by Politzar and co-workers [21] to be improved by reducing the radiation dose from 15 krad to 11 krad, but even at the lower dose the lifespan was well below that of wild males. It was concluded that this was principally due to irradiation and secondarily to the particular conditions of rearing at the time of the experiment [21].

It has been argued that reduction in lifespan of released males would not reduce the efficiency of a programme because male tsetse are physiologically capable of exhausting their lifetime's supply of sperm within 10 days. Furthermore, tests of the quality of sterile male tsetse and other insects frequently involve checking whether they are capable of the normal number of inseminations if provided with many virgin females. However, in the authors' opinion it is a serious mistake to overlook the fact that a female tsetse (like many other Diptera) is only receptive to one or a very few inseminations, and consequently, when there is a 1:1 sex ratio, but especially when releases have created an excess of males in the mixed population, each male will seldom encounter a receptive female.

Thus numerous matings by any one male are very improbable and to maximize the chances that a sterile male will encounter even one receptive

female in its lifetime, it is important that it has as long and active a life as possible.

In *G. morsitans*, as in many other organisms, the use of an atmosphere of nitrogen during irradiation has a protective effect against radiation-induced damage. Both the genetic (sterility) and somatic (reduced longevity) effects are less for a given dose if a nitrogen atmosphere is used rather than air [19]. However, the protection against somatic damage is more than against genetic effects so that lifespan is longer at the dose in nitrogen required for 93% sterility (for example) than at the lower dose in air required for the same sterility [19].

Heavily irradiated insects show behavioural abnormalities ('radiation-induced lethargy'). Measurement by Brady of seven behavioural parameters in the laboratory showed significant deviations from the norm in four of them for *G. morsitans* males which had been given 12 krad in air but, after the dose in nitrogen required to give the same sterility, the flies deviated from the norm in only one parameter [22]. This may indicate that mating competitiveness in the field would be better if nitrogen anaesthesia were used during irradiation. However, the authors are not aware of published data on this point. Clair and co-workers [20] working with *G. palpalis* and the high dose of 15 krad in air give data on the released : wild male ratio and the amount of sterility induced in the wild females. It is possible to calculate from these data that competitiveness of the sterile males was only about 16% of normal [23]. It will be interesting to see corresponding calculations for more moderate dosages and dosages given in nitrogen.

In the case of vectors of human trypanosomiasis such as *G. palpalis* it has been the policy of the team working in Upper Volta to irradiate adults rather than pupae. This is done to minimize the risk that the releases could add to the chances of transmission of the human disease [24]. These chances can be minimized by releasing adults after they have received at least one trypanosome-free blood feed, as the *Trypanosoma brucei* group are thought to be unlikely to infect flies except at their first feed [25]. Since the releases are not made until the post-teneral stage it is logical to delay irradiation until this stage as the later in the life cycle that insects are irradiated, the less the somatic damage that is caused. The authors recently carried out a small-scale test of irradiation of young adult *G. morsitans* and *G. palpalis* in nitrogen or in air. The results have been recorded in terms of survival, fertility and responsiveness of the males to light. In *G. morsitans* there was no evidence for any reduction in responsiveness to light or survival up to 33 days after 15 krad in nitrogen or 11 krad in air. However, with *G. palpalis* survival seemed to be better after the former treatment than after the latter (Table II). Both irradiation treatments reduced responsiveness to light (Table II). There was no significant difference between the effects of the high dose in nitrogen or low dose in air in this respect ($\chi_1^2 = 0.48$). About the same degree of sterilization seems to have been achieved by each radiation treatment. However, much more data would be required to check that these

TABLE II. EFFECT OF IRRADIATING ADULT *G. palpalis* MALES IN AIR OR NITROGEN

Irradiation	Percentage responding to light	Percentage survival to day 33	Fertility of mates
15 krad in N_2	55	75	0.026
11 krad in air	49	26	0.028
Unirradiated	71	82	0.446

Notes: Ten tenerals and 10 males aged 4 days were given each treatment. Response to light was tested by darkening the cage and counting the flies exiting into a well-lit tube: the test was carried out weekly for 5 weeks.
Fertility was measured as pupae/(surviving females X ovulation cycles).

two treatments really do give equal sterility and therefore allow a fair test of whether the use of nitrogen is beneficial when irradiating adults.

In *G. moritans*, which is of importance mainly as a vector of animal (*T. vivax* and *congolense)* trypanosomiasis, it is considered important to release in such a way that flies are not confined even for a few hours after emergence, otherwise wing muscle development was found to be abnormal by Bursell and Kuwenga [26]. The ineffectiveness of one experimental release programme was attributed to confinement of the males after emergence [27]. There would appear to be a difference between *G. morsitans* and *G. palpalis* in this respect, or between the precise caging conditions used by different teams of experimenters because Politzar and co-workers [21] have shown almost normal dispersal of *G. palpalis* released in Upper Volta at the adult stage.

Working on the assumption that it is necessary to release *G. morsitans* before emergence, almost all the irradiations of this species have been at various times in the puparial stage [12, 19, 22, 28, 29]. Irradiation of young pupae is lethal, and this means that radiation sterilization of puparia collected in the field is wasteful, as the ages are inevitably mixed, the younger ones cannot be distinguished by external inspection and are killed on irradiation. Puparia of known age from captive colonies can be irradiated just before emergence. This has the great advantage that the females, which emerge before most of the males, can be separated off before sterilization, thus providing the necessary large input into the captive colony of breeding females (together with the necessary small proportion of fertile males to inseminate them), and it also avoids the release of almost all the females, which would add unnecessarily to the vector population.

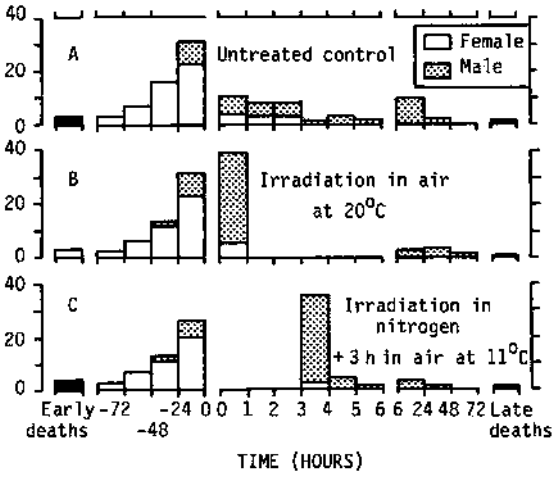

FIG.5. Normal emergence pattern of G. m. morsitans *from puparia (A), the effect of irradiation in air (B), or in nitrogen followed by three hours' maintenance at 11°C (C). Note that the time scale is expanded in the middle. Data from Ref.* [*19*].

Irradiation of puparia in air in the last 24 hours before the time at which they are due to emerge has the effect of stimulating immediate emergence [30]. The mechanism of this effect is unknown but, at that stage, emergence can be triggered by merely pricking the puparium with a pin. Emergence stimulated at irradiation would mean that adult flies would have to be caged and transported to the release site, and failure of normal wing muscle development would therefore presumably occur. An atmosphere of nitrogen during irradiation temporarily inhibits the stimulation of emergence. If the puparia are transferred from the nitrogen atmosphere to air at about 11°C they may be held there for at least 3 hours for transportation. On re-warming they 'remember' that they had been irradiated and stimulated emergence occurs of almost all those due to emerge within the next 24 hours (see Ref. [19] and Fig.5). Emergence immediately on arrival at the release site gives an improved chance of escape from mortality due to predators or solar heating. With this technique, releases must be made within a few hours of irradiation, but daily releases can be avoided if this is inconvenient by accumulating at 11°C up to 5 days' batches of puparia after female emergence has been completed and before irradiation [22]. This composite technique of accumulation at 11°C before irradiation, irradiation in nitrogen and transport to the release site at 11°C was used successfully for 18 months in the release programme in Tanzania [29].

Almost all the research on radiation sterilization had been with gamma rays, but recently Hamann and Iwannek [31] have reported the use of beta rays

from sources which can safely be shielded with only 1 cm of plastic. Five krad were required for 98% male sterilization but survival appeared normal up to 10 krad.

CHEMOSTERILIZATION

In experiments with the aziridine compound tepa Dame and Ford [32] obtained full and permanent sterility of either sex by injection, tarsal contact with a deposit on glass or by dipping of puparia. Survival of treated males was slightly sub-normal but competitiveness in laboratory tests was good. The pupal dipping method appears to have its effect as a result of contact of the adult with the outside of its puparium during the act of emergence. This was shown by the fact that dipped and subsequently washed pupae did not give rise to sterile adults. Pupae dipped three weeks before emergence do not show full sterilization, apparently because of decay of the chemosterilant deposit on the puparium. Nevertheless the dipping method with wild collected puparia of mixed ages proved successful in a field trial on an island in Lake Kariba, Zimbabwe [27].

The main difficulty in the sterile insect release method for tsetse flies is, of course, mass rearing sufficient numbers. The autosterilization concept [33] in which members of the wild population are sterilized and then allowed to return to the population is therefore very attractive. Chemosterilants can certainly not be spread broadcast in the environment, and it is therefore necessary to attract the flies to a protected source of chemosterilant. Vale and co-workers [34] have developed odour-baited traps of high efficiency, and equipped these with a chamber in which trapped flies are automatically exposed to an aerosol of metepa for a standard time before automatic release. The device attracts and sterilizes both males and females. The sterilization of the females may be considered approximately equivalent to killing them (except that they are not immediately removed from the vector population), but the sterilized males can pass their sterility on to mates which did not encounter the traps. The sterilizing traps are currently being field tested on the same island as the earlier tepa-sterilized release experiment (G.Vale, personal communication). Before extending their use on a wider scale the question of securing the unattended metepa spray cans against theft, which would be highly dangerous, will have to be considered and the cost of this will have to be weighed against the somewhat less efficient use of the traps simply to kill the flies attracted into them.

Hamann and Iwannek [31] are studying the use of beta-ray sources as an alternative to chemosterilants for autosterilization.

The safety of an autosterilization method could be greatly improved by the use of extremely small localized deposits of chemosterilant associated with decoys baited with contact sex pheromones [35]. A 'following swarm' of male

TABLE III. FERTILITY REDUCTION IN MALE *G. morsitans* FOLLOWING COPULATION FOR DIFFERENT PERIODS OF TIME WITH RECTANGULAR NYLON DECOYS BAITED WITH 100 μg SEX PHEROMONE AND DOSED WITH DIFFERENT AMOUNTS OF THE CHEMOSTERILANT BISAZIR [37]

Bisazir dose ($\mu g\ cm^{-2}$)	Exposure duration (h)	Male fertility ÷ control
100	0.5	0.418
100	1.0	0.068
100	1.5	0.077
100	2.0	0.038
320[a]	0.5	0.030
320[a]	1.0	0

[a] Maximum dose at which males are not irritated and 100% of test flies remain in copulating position for at least 0.5 h.

G. morsitans can be visually attracted in the field with a simple square of black cloth where individuals are further visually stimulated to make contact with pheromone-baited decoys attached to the cloth and will attempt to mate with them [36]. The thioaziridine compound bisazir has been shown to be effective in the laboratory as a sterilant which can be picked up by males in sufficient quantity to induce sterility during the act of attempted copulation with the decoy [37]. All test males in the laboratory indulge in copulatory activity with pheromone-baited decoys (100 μg or 25 female equivalents) for at least 30 minutes, and the duration of response is unaffected by the presence of bisazir up to a concentration of 320 $\mu g\ cm^{-2}$ (Table III). The response duration in a field test in Zimbabwe was no more than 2 minutes, but the pattern of response duration was unaffected by bisazir concentrations as high as 1200 $\mu g\ cm^{-2}$ (Langley, Coates and Carlson, in preparation). Hence it is likely that much higher concentrations could be used. An exposure time of 2 minutes would require a dose of 3600 $\mu g\ cm^{-2}$ to produce 90% sterility, but the dose of 1200 $\mu g\ cm^{-2}$ would be expected to produce 50% sterility in 2 minutes. The technique, of course, has no direct sterilizing effect on the wild females. Its efficiency is improved by combining a visually attractive cylindrical model and the olfactory stimulation of CO_2 and acetone to attract very large numbers of flies [38]. Approximately 24% of males attracted to such a model actually engage in copulatory activity with the decoys upon it.

Hence baited traps or decoys, coupled with chemosterilization, seem to have a great potential for tsetse control. It should not be overlooked, however, that the percentage of the population affected by them will remain constant as the population declines and eradication by this means may be slow. It could be that the final 'mopping up' of the residual population left by this type of method could best be done by some releases of sterile males which will benefit from the 'Knipling effect' of sterilizing a progressively higher percentage of the wild population as this population declines [33]. One should also not overlook the possibility that resistance to autosterilization could evolve, either by selection for non-responsiveness to the bait or for resistance to the chemosterilant. Resistance to chemosterilants has been selected in the laboratory in mosquitoes after many generations of exposure [39, 40]. Such a process is no hazard where the insects for treatment and release are taken out of a breeding stock which is not itself exposed to chemosterilant, but the situation is different where a segment of a wild population is treated and any non-responders could form a considerable proportion of the fertile breeding stock which will produce the next generation.

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A MODIFIED WING-FRAY ANALYSIS TECHNIQUE FOR AGE ESTIMATION IN *Glossina*

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Abstract

A MODIFIED WING-FRAY ANALYSIS TECHNIQUE FOR AGE ESTIMATION IN *Glossina*.

An important shortcoming of the wing-fray analysis technique in current use for estimating the age of male and female *Glossina* is that no distinction is made between teneral and young non-teneral flies. This limits the use of the technique for the entomological evaluation of tsetse control operations in which it is desirable to have a single technique for determining teneral percentages and the overall age structure of a population sample. A modification of the original wing-fray method is described which overcomes this shortcoming. Instead of allocating individual flies to one of six wing-fray categories (numbered 1–6), they are allocated to one of seven categories (1A, 1B, 2–6). Previously, category number 1 contained both teneral and non-teneral flies, but with the modified method those flies are allocated to categories 1A and 1B respectively. Similarly for the calculation of mean wing-fray values and for the preparation of age structure graphs, category multiplication factors X1–X7 replace those of X1–X6. The modified method has been successfully applied in the field for studies of *Glossina papalis* and *G. morsitans centrâlis* populations which were subject to tsetse control measures.

INTRODUCTION

During the evaluation of any tsetse control operation which depends on the application of chemical insecticides or on the mass release of sterile male insects, it is important to estimate, as precisely as possible, the age of flies captured before, during and after the control activity.

A reasonably accurate estimation of female age can be obtained by the ovarian dissection method [1], although the technique is tedious and time

TABLE I. ORIGINAL JACKSON WING-FRAY METHOD APPLIED TO A SAMPLE OF *G. m. centralis* COLLECTED IN MUMBWA DISTRICT ON 14 MARCH 1980

W.-F. Category	1		2		3		4		5		6		Totals	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
a. No. of flies	55	47	46	71	45	69	30	17	14	8	8	4	198	216
b. Percentages	27.7	21.7	23.2	32.8	22.7	31.9	15.1	7.8	7.1	3.7	4.0	1.8	99.8	99.7
c. Multiplication factor	X1		X2		X3		X4		X5		X6		–	
d. Calculation (a X c)	55	47	92	142	135	207	120	68	70	40	48	24	520	528
e. Mean W.-F. values													2.6	2.4

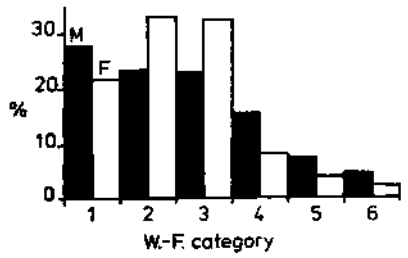

FIG. 1. Bar graph presentation of the data given in Table I. Note the high values for category number 1.

consuming. An estimate of male age can at present only be obtained by measuring the degree of wing fray [2], a technique which is rapid and easily mastered by junior technical personnel. Because of these anomalies many tsetse workers find it more convenient to use the wing-fray technique for both male and female flies, irrespective of whether or not the ovarian dissection method is employed.

Unfortunately, because the first wing-fray category (number 1) can be applied to both teneral and some young non-teneral flies, wing-fray analyses of population samples do not reflect the proportion of teneral flies in a sample. A separate calculation is necessary to determine the percentage of teneral flies.

The authors describe a modification of the original wing-fray analysis technique which was developed for studying populations of *Glossina palpalis* in the Ivory Coast, and which has subsequently been successfully applied to populations of that species in Nigeria, and to *G. morsitans centralis* in Zambia. The modified technique provides more meaningful data on population age structures without having to make a separate calculation of the teneral/non-teneral ratio.

ORIGINAL WING-FRAY METHOD

An example of the original wing-fray method [1] applied to a Zambian sample of a *G. m. centralis* population is presented in Table I and Fig.1. It should be noted that in neither presentation is it possible to distinguish between teneral and non-teneral flies in category 1. Furthermore, the upper margin of the bar graph shown in Fig.1 does not follow a smooth curve.

TABLE II. MODIFIED WING-FRAY METHOD APPLIED TO THE SAME DATA AS PRESENTED IN TABLE I

W.-F. Category	1A		1B		2		3		4		5		6		Totals	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
a. No. of flies	24	15	31	32	46	71	45	69	30	17	14	8	8	4	198	216
b. Percentages	12.1	6.9	15.6	14.8	23.2	32.8	22.7	31.9	15.1	7.8	7.1	3.7	4.0	1.8	99.8	99.7
c. Mult. factor	X1		X2		X3		X4		X5		X6		X7		–	
d. Calculation	24	15	62	64	138	213	180	276	150	85	84	48	56	28	694	729
e. Mean W.-F. values															3.5	3.4

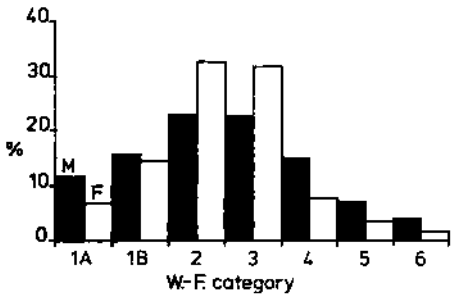

FIG.2. Bar graph presentation of the data given in Table II. Note the smoother margins of the curves resulting from distinguishing between teneral (category 1A) and young non-teneral flies (category 1B).

MODIFIED WING-FRAY METHOD

With the modified method flies are allocated to one of seven categories instead of six, and the multiplication factors have been changed. Comments on the seven categories are as follows:

Category	*Comments*
1A	Only teneral flies. Equivalent to part of the original category 1. (Mult. factor 1.)
1B	Non-teneral flies with completely clear and intact wings. Equivalent to part of the original category 1. (Mult. factor 2.)
2–6	Equivalent to the original categories 2–6. (Mult. factors 3–7 respectively.)

Table II and Fig.2 show how the modified method has been applied to the same *G. m. centralis* sample as was used to illustrate the original method of analysis.

Apart from the distinction between teneral and non-teneral flies, it is also obvious that the margin of the Fig.2 bar graph presents a much more normal type of curve which permits a better appreciation of the non-teneral component of the sample.

It will also be noticed that the mean wing-fray values obtained by the two methods are quite different, but those differences are of no consequence because the values themselves are meaningless until they are converted to calendar age by appropriate conversion factors.

DISCUSSION

The advantages of the modified wing-fray method are sufficiently obvious not to require further detailed discussion. However, it is pertinent to stress the value of being able to distinguish between teneral and young non-teneral flies. This applies particularly to studies of tsetse populations which are in contact with insecticide deposits on vegetation, and where it is important to determine the break-off point of persistence efficiency. It is equally applicable to studies of tsetse populations composed of wild and sterile individuals, where it is important to know (a) whether the pupal reservoir has been exhausted, and (b) whether, due to an incorrect wild/sterile ratio or to re-invasion, there has been a resumption of breeding.

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PROSPECTS FOR USING STATIONARY BAITS TO CONTROL AND STUDY POPULATIONS OF TSETSE FLIES (Diptera: Glossinidae) IN ZIMBABWE

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Abstract

PROSPECTS FOR USING STATIONARY BAITS TO CONTROL AND STUDY POPULATIONS OF TSETSE FLIES (Diptera: Glossinidae) IN ZIMBABWE.

Refined methods of studying the behaviour of tsetse flies, *Glossina morsitans morsitans* Westw. and *G. pallidipes* Aust., have indicated the possibility of producing stationary baits that can attract up to about a hundred times as many tsetse flies as can be caught from the types of stationary baits and capture systems commonly employed in the past. Three types of refined bait may be of value for controlling and/or sampling tsetse populations: (1) simple targets to expose the attracted flies to insecticide, (2) traps to retain the flies and (3) traps to capture, sterilize and release the flies. The economics of employing these baits, and the potential benefits of adding odour attractants to them, are discussed. Compared with conventional methods of tsetse control, bait methods can have the advantages of avoiding (1) risks of environmental pollution, (2) demand for technological sophistication, (3) uneven use of labour and equipment throughout the year, and (4) difficulties of timely correction of errors. Several research projects associated with the development of odour-baited sterilizing traps in Zimbabwe are reviewed. It is shown that effective attractants in ox odour occur in the breath; carbon dioxide and acetone may account for some, but not all, of the attractiveness of the breath. New methods to assay odour attractants in the field are being developed using techniques that measure the recruitment of flies from upwind and downwind of test sites. An analytical approach to the trap-orientated behaviour of tsetse has led to the development of an efficient trap. An automatic device has been constructed to place on a trap to sterilize the captured flies with a metepa aerosol and release them. A small field trial of odour-baited sterilizing traps has been started with an isolated population of tsetse flies on an island in Lake Kariba.

INTRODUCTION

In the last decade, three discoveries have enhanced our understanding of the numbers of tsetse flies, *Glossina morsitans morsitans* Westw. and *G. pallidipes* Aust., that are potentially available to baits in the field. First, the use of efficient electrocuting traps has shown that handnets and conventional trapping systems

fail to catch many of the tsetse that visit baits [1–3]. Second, the common expedient of placing a man next to a bait to count or catch the attracted flies has been found to reduce greatly the numbers of tsetse, females especially, that come to the bait [1, 2, 4]. Third, most of the tsetse that arrive at stationary hosts in the field have been shown to arrive in response to host odour; large doses of such odour can attract huge numbers of tsetse [1, 5]. These discoveries have indicated that stationary baits can be produced that will attract up to about a hundred times as many tsetse as can be caught from the types of stationary baits and capture systems commonly employed before 1970. Thus, our assessments of the opportunities for the practical applications of baits have improved enormously. These opportunities are discussed and some of the current work to develop the use of baits in Zimbabwe is described.

TYPES OF PRACTICAL APPLICATION

There are several ways of dealing with the flies that arrive at a stationary bait. The flies might not be captured, but simply killed, perhaps by contact with insecticide deposited on the baits. Provided the density of the distribution of such baits, and their individual performances, were sufficient to kill about 2.5% of the female population daily, the baits would be highly effective for tsetse control; odour-baited devices at densities of only one per few square kilometres can achieve this percentage catch [6].

Alternatively, the flies might be captured and retained in traps. As a measure for tsetse control, a trap that captured a high proportion of the attracted flies would be as effective as the insecticide-treated target. However, traps have the distinct advantage that the numbers of flies retained can be used as indices of the abundance of tsetse, so that trapping provides an integrated operation to control and monitor tsetse populations. This could help solve the long-recognized problem of obtaining adequate samples of tsetse in areas where the populations of tsetse are exceedingly sparse. In this context, it is particularly noteworthy that traps, unlike many other sampling systems, can provide satisfactory samples of each sex, species and age group of wild populations [7]. Even for control operations based primarily on insecticide-treated targets, it would be useful to employ monitoring traps at a few stations.

Retaining traps have exciting prospects as research tools, particularly in regard to mark, release and recapture operations. The use of just one improved trap can recapture in a single day about 8% of the marked flies released [6], so that a field entomologist armed with several traps could expect to have his marked flies on almost instant recall. This facility has potentially important applications in many types of study. For example, to study the effect of the exposure of known flies to insecticide in the field, it would be pertinent to

measure the recapture rates of marked flies released before and after the application of insecticide to an area around the release point. A potentially important advantage of such a technique is that invasion of the treated area by unmarked flies would be irrelevant, so that the treated area could be small, say a few square kilometres, whereas the usual methods of evaluating a control measure, which are based on the changes in catch levels of unmarked flies, require the treatment of several hundred square kilometres to minimize invasion of the treated area [8].

The most refined use of traps in tsetse control operations would involve the capture, sterilization and automatic release of the trapped flies. The females released would be effectively dead and the males released would move from the trap to interfere with the reproduction of females with which they subsequently mated, so that a sterilizing trap would be about twice as effective as a retaining trap. A useful refinement would be to ensure that a proportion of the catch was diverted to a retaining cage, to provide indices for the numbers of flies passing into the sterilizer and for changes in tsetse abundance.

ADVANTAGES OF BAIT METHODS OF TSETSE CONTROL

Tsetse control based on one of the various types of bait could avoid some of the problems associated with those conventional methods of tsetse control that involve the management of host populations [9] or the extensive use of insecticides [8]. Such problems can include the risks of broad ecological change, logistical difficulties and the demand for a high level of technological sophistication.

A particularly difficult problem with the normal use of insecticides is that these can be applied satisfactorily during only a few months of the year, which presents an uneven demand for staff and equipment throughout the year, and allows tsetse populations to recover by reproduction and invasion during those months when the insecticide has become ineffective. Compared with this, the steady inputs associated with the construction, placement and routine servicing of baits, and the year-round efficacy of the baits, present distinct advantages. Moreover, the steady, cumulative effect of employing baits means that errors in the use of one of many baits in, say, one month, can be corrected next month with no serious loss to the efficacy of the control programme. This situation should be contrasted with that in which one cycle of an aerial spraying operation with non-residual insecticide has gone awry due to, say, unsuitable weather for applying the insecticide. Such an occurrence could severely prejudice the ability of the whole spraying programme to eradicate the flies; the correction of this fault could be exceedingly expensive, and might not be possible before the end of the spraying season.

COST AND EFFECTIVENESS

Bait methods of tsetse control offer some clear technological and administrative advantages, but whether or not the baits are, indeed, feasible alternatives to conventional methods of tsetse control, and whether or not they should be used with or without odours, and be adapted to insecticide treatment, or trapping and retention, or trapping, sterilization and release, are largely matters of cost and effectiveness.

The relative effectiveness of insecticide-treated targets, retaining traps and sterilizing traps would be roughly 1:1:2 respectively. The relative cost of making and repairing the three types of devices would be much more discrepant, perhaps 1:4:16 respectively, but the costs of manufacture and repair are likely to be only a small part of the overall costs of operating each device. Such overall costs would include the expenses of inspection, transport and administration, and might be much the same per bait station irrespective of the type of bait employed. Hence the overall cost of operating a cheap insecticide-treated target might not be grossly less than the overall cost of operating the much more expensive, and substantially more effective, sterilizing trap.

Large doses of natural odour can enhance by about 20 times the numbers of flies arriving at stationary baits [5] so that, provided odours can be dispensed at each bait station for costs that are not many times greater than the overall cost of operating the station without odour, it would be economical to employ the odours and to use far fewer stations than would be required for odourless baits. The methods now used to dispense natural odours [2] are far too expensive and impractical for extensive use. To improve the economy and convenience of employing odour attractants, it is necessary to identify the effective components of natural odour and to dispense them artificially.

RESEARCH

A more detailed assessment of the economics of the various types of bait for tsetse control awaits investigations of the ways in which the baits may be refined, and of field trials of their performances. Such research, now in progress, is aimed at the types of bait at each extremity of the range of technological options: on the one hand studies are being made of the use of a high density of low-cost, odourless targets for insecticide treatment, and on the other hand investigations are being conducted into the use of a low density of relatively expensive, odour-baited traps for sterilizing operations.

Research into the development of odour-baited sterilizing traps is being given most attention, partly because such traps are the most elegant and potentially the most effective forms of bait, but also because if these traps can be produced

satisfactorily, several of the simpler types of trap could be developed from them with relative ease. Production of the sterilizing traps involves the several distinct projects described below.

Odour identifications

Some initial clues for the identity of the attractive odours were produced by studying the efficacy of odours from different sources. Odours from many different hosts were tested in the field by hiding the hosts in a covered pit and expelling air from the pit to ground level. Flies attracted to the odours were captured by an electrocuting trap. Alternatively, the hosts were placed in a pen at ground level and the importance of their odour as an attractant was assessed from the proportion of flies that approached the pen from downwind [2]. With oxen, donkeys, goats, sheep, buffalo, kudu, bushbuck, impala, bushpig, warthog and chickens, the attractiveness of the odour of each host appeared to depend on body size rather than on host species per se. This suggests that the odour is a chemical, or combination of chemicals, common to the odours of many animals, and that the quantity of the chemical released is dependent on body size. An apparent anomaly in this matter was provided by men who, despite their fairly large size, produced an odour that attracted very few flies; in fact human odour, especially body odour, appeared repellent, for when mixed with ox odour it reduced the efficacy of ox odour [10]. Perhaps men produced the same type of attractive odour as was produced by other animals but its effect was masked by repellents.

Injection of an ox or goat with xylazine (Rompum: Bayer Agrochem), a sedative that reduces the metabolic rate, reduced the efficacy of odours from these animals by about half [11]. This suggests that the rates at which the attractants are released are closely geared to the metabolic rate at the time of release, as against, say, being slowly released from accumulated residues on the body surface. This is confirmed by the indication that a freshly washed ox produced the normal level of odour attraction [2].

Feeding an ox on roughage alone, or on concentrated food alone, for the few hours in which its odours were collected and assayed, produced no clear effect on the efficacy of the odour [2], but long-term changes in diet did produce marked effects. During a week of starvation, the efficacy of the odour generally declined slightly, but subsequently, during the first few days on a fattening diet, it rose sharply, to be followed by a series of diminishing peaks and troughs in the efficacy of the odour. Thus, the important components of the odour do not appear to be enhanced by the presumed mobilization of fat and the mild acetonaemia at the end of the starvation period. Rather they appear to be associated with the revitalization of the digestive process and metabolism at the start of fattening. The rumenal gases of an ox on a normal diet appear unattractive [2].

Further clues to the identity of odour attractants were produced by two types of approach: first, attempts to produce progressively purified fractions of natural odour, and second, empirical tests with known chemicals.

Regarding the fractionation of natural odour, the head odours of an ox or donkey produced an odour as effective as the whole odours of these animals, whereas body odours excluding those from the head were poorly effective [2]. The breath of an ox was almost as effective as the whole-head odours, but the odours from the surface of the head of a live ox, or from the whole of the head removed from a slaughtered ox, attracted very few tsetse. Carbon dioxide released at roughly the rate at which it is released in ox breath was mildly attractive, but insufficiently so to account for all of the odour attraction to an ox [10]. Passing the ox breath through dilute acid, to remove bases, did not destroy its efficacy. Passing the breath through soda lime, to remove carbon dioxide and acids, greatly reduced but did not completely destroy its efficacy. When soda-lime-treated breath was mixed with carbon dioxide, it attracted more flies than were attracted to carbon dioxide alone or to the soda-lime-treated breath alone. It appears that the breath of oxen contains a neutral substance, or combination of such substances, which are only mildly attractive unless dispensed with carbon dioxide. The effective components of ox head odour have been trapped in, and regenerated from, a condenser immersed in liquid air, but it has been impossible so far to trap and regenerate them using organic solvents.

Regarding the empirical approach, a wide range of chemicals has been studied for their olfactory activity when dispensed alone or with carbon dioxide [10, 12]. Ketones and aldehydes with short aliphatic chains, such as acetone and propionaldehyde, appeared attractive, whereas such compounds with long aliphatic chains, such as n-heptaldehyde, or with aromatic rings, such as acetophenone, appeared repellent. Lactic acid and caproic acid were also repellent.

The artificial odour that has been investigated most is a mixture of carbon dioxide and acetone. With carbon dioxide at 6 litres/min and acetone at 1 g/min the odour was roughly as effective as the natural odour of a large ox. However, these doses of carbon dioxide and acetone are far greater than the doses at which they occur in ox odour. It is necessary to study the performance of carbon dioxide and acetone mixed with the other attractants that have been identified, and to continue the search for attractants that have not been identified.

It is unfortunate that carbon dioxide is required to maximize the efficacy of the other attractants that have been discovered so far; if carbon dioxide must be used to potentiate the artificial odours released near traps, the cost and inconvenience of the gas cylinders and flow regulators would detract from the economics of dispensing the odours. However, the cost of dispensing the carbon dioxide would not necessarily be prohibitive. Moreover, it is encouraging that acetone alone at a few grams per hour can increase the catches from traps by several times [12], offering some reason to hope that when all of the components

of natural odour have been identified, the release of a mixture of large doses of these chemicals will produce good attraction in the absence of carbon dioxide.

Further work to identify attractants is planned as a three-pronged approach: (1) chemical fractionation and antennagram work by the Tropical Products Institute, London, (2) laboratory assays of attractants by the Tsetse Research Laboratory, Bristol, and (3) field assays by the Branch of Tsetse and Trypanosomiasis Control, Zimbabwe.

Field methods to assay attractants

In order for Zimbabwe to fulfil its part in the further identification of odour attractants, it is necessary to discover more precise methods of assaying odour attractants in the field. Ideally a technique is required that will allow the accurate assessment of small quantities of odour in only one or a few replicates of the test. Current methods usually require the release of large quantities of odour next to a trap, followed by comparison of the catches of this trap and the catches from control traps treated with no odour or with a standard odour. Even when the studies eliminate the contributions of site and time to the variance of the data produced, the residual variance usually remains so great that a test odour has to produce in many replicates a mean catch that is at least half or at least double the control catch before the effect of the test odour becomes clear [12].

The requirements for improved test procedures are essentially of two types: (1) the need to enhance the efficacy of the conditions under which the odour is released, so that even small doses of test odour will increase greatly the index of odour attraction relative to this index for the control, and (2) the need to reduce substantially the residual variance of the indices.

Regarding the relative increase of the index for the test odour, preliminary work suggests that the numbers of flies that can be recruited to a trap by carbon dioxide and acetone can be increased by releasing these attractants in small doses at 2 m intervals, downwind of the trap for 64 m, instead of the usual expedient of releasing these attractants in one large dose next to the trap. Presumably the long thin plume of odour that results from the amalgamation of the many small plumes, extends further than the broader plume that is produced from the single release point. Other work suggest that there is merit in employing a small plume of test odour that extends downwind from the trap to a very large source of standard odour; in the presence of an effective plume of the test odour, the large numbers of tsetse attracted to the standard odour have been shown to transfer to the source of the test odour. Thus, a small bridge of test odour might be used to trigger the capture of large numbers of tsetse.

Regarding the reduction of the residual variances, the main cause of a high residual variance for conventional methods of odour testing appears to be that the availability of tsetse at a particular site on a particular day is poorly correlated

with the availability of the flies at another site on that particular day, so that, for example, an odour that is usually highly effective can, on one day at one site, produce a catch that is exceedingly poor in relation to a simultaneous control catch at another site. One method that was designed to overcome this problem was to ensure that all tsetse that were potentially available to the test odour were those that had recently flown into that area where they could be recruited to the test odour; the numbers of flies actually recruited to the test odour in each replicate were then compared with the numbers that were estimated to have entered the area of potential recruitment. Unfortunately, this technique was unsatisfactory for it required the recruitment area to be cleared of the vegetation on which tsetse rest, and this reduced greatly the numbers of flies that flew into the area [13].

Another approach is to employ the test odour and the odourless control at the same site on the same day, and to express the catch with the test odour as a proportion of the catch with no odour. To further this approach, tests are now being made with a variety of devices involving the release of odour near a visual target, with a strict separation between catches of flies recruited from upwind and downwind, so that the downwind catch relative to the upwind catch indicates the effect of the test odour. This type of approach has already been employed with the bait pen discussed above, but with the pen it was crude since the pen, unlike the new devices, could not adjust its orientation to changes in the wind direction during each replicate.

Trap improvement

Traps are required to capture all of the tsetse attracted to them by odours. To ensure that the males that are sterilized and released have a good chance of survival, it is important that they do not deplete their energy reserves during the trapping and sterilizing process. Thus the tsetse arriving near the trap should be caught rapidly and, when in the trap and awaiting release, the tsetse should not be activated by disturbance from other trapped insects. Conventional traps capture no more than about half of the tsetse attracted to them, the tsetse are not caught rapidly and the catches contain many flies other than tsetse [3, 14]. Clearly, it is necessary to design better traps.

A rational attempt to improve traps required a study of the trap-orientated behaviour of the tsetse and other insects. Such studies recognized several distinct types of behaviour in the sequence of responses leading to trapping. Using various electrocuting devices to catch insects in or near a variety of trap-like objects, studies were made of the features of trap design that affected each type of response. These responses, and some of the design features that influenced them in the required manner, are discussed below.

Tsetse arriving within a few metres of the trap are required to move close to the trap. A wide variety of objects, either on or off the ground, cylindrical or conical, large (1 m^3) or small (0.1 m^3), and black, white or striped, were suitable to induce a strong approach reaction.

Having arrived close to the trap, the tsetse are required to enter the trap. Entrances restricted to the base were effective for encouraging the entry of tsetse and tended to discourage the entry of Stomoxyinae, non-biting Muscidae and Tabanidae. By making the outside of the trap white over most of its surface, with black near the entrances, alighting flies concentrated near the entrances and the entry response was enhanced. Tsetse entered most readily when the inside of the trap, seen from the entrances, was white rather than black.

Once the tsetse has entered the trap it must not return through the entrances but must move to the top of the trap where a light-seeking response takes it into a cone of netting that guides it into a retaining cage. One means of reducing return flights through the entrance was to provide the entrance with a tunnel, 20 cm long, that projected into the trap. Tsetse moved up to the cone if the inside top of the trap was made black, to contrast with white at the inside base. Tsetse near the top black target could be induced to seek light by ensuring that they collided with fine black netting while flying towards the black target.

Those tsetse entering the base of the cone are required to move straight to the top of the cone. This movement was encouraged by placing an open-ended tubular shade around the cone so that the only light apparent to the flies was from the top of the cone, rather than from the top and the sides.

Having arrived at the top of the cone, tsetse must transfer to the retaining cage and remain in this cage until sterilized and released. An opening of 5 X 10 cm, leading from the top of the cone to a cage 50 X 25 X 25 cm, was suitable to induce virtually all tsetse to transfer from the cone to the cage and to remain in the cage for at least 30 min.

A variety of traps was designed on the principles suggested above, and taking into consideration the ease of eventual mass production, erection and operation. The shape of all these traps was an upright triangular prism, the sides of which could be formed simply by stretching a length of cloth around three upright poles supported by guy ropes. Another benefit of the triangular shape is that the retaining cage can be placed near one corner of the trap where it is readily accessible for inspection and where it can be fitted with sterilizing accessories that are well clear of the trap. The entrances to the trap were triangular since such entrances and their tunnel-like devices, could be made simply by tucking in part of the wall of the trap. One of these traps, termed the beta trap, was found to perform well; it is shown in Fig.1A.

Using carbon dioxide and acetone as odour attractants, the catches from a beta trap were compared with those from a white biconical trap and from a blue biconical trap since biconical traps are in common use in many parts of Africa [15].

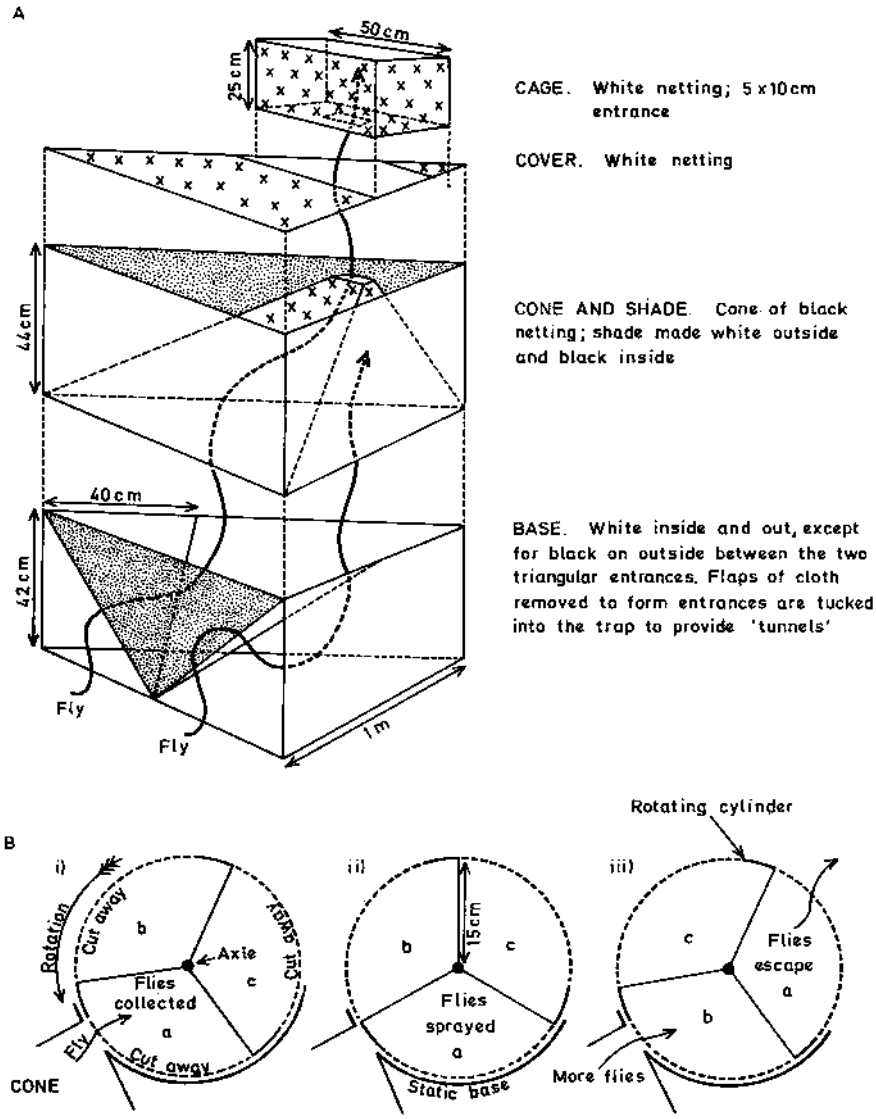

FIG.1. A. Exploded view of a beta trap; B. Cross-section of the sterilizing device, in three stages of the rotation of the cylinder.

There was no clear difference between the catches from the two biconical traps, but the catches from the beta trap contained consistently more tsetse, and consistently fewer Stomoxyinae and non-biting Muscidae than occurred in the catches of the biconical traps. Catches from the beta trap as a percentage of the catches of each biconical trap, averaged 274 for *G. morsitans*, 214 for *G. pallidipes*, 24 for Stomoxyinae and 4 for non-biting Muscidae.

The catches ffom one beta trap were compared with those from another beta trap at which the attracted flies were captured efficiently by electrocuting devices soon after the flies had arrived near the trap, so that the catches of the beta trap alone, as a percentage of the catches of the beta trap plus electrocuting devices, indicated the efficiency with which the beta trap alone captured flies attracted to it. These percentages were 100 for *G. pallidipes*, 51 for *G. morsitans*, 6 for Stomoxyinae and 1 for non-biting Muscidae. Other work suggested that tsetse entered the cage of the beta trap about 5 minutes after arrival near the trap.

The beta trap appears satisfactory for field trials of sterilizing traps, but clearly its performance is far from perfect, especially in regard to its relatively poor efficiency for *G. morsitans*. Further attempts to improve trap design are in progress.

Sterilizing devices

A sterilizing and releasing device (Fig.1B) has been designed to replace the retaining cage of the beta trap. The device consists of a revolving cylinder of clear perspex with three similar chambers. In the position of Fig.1B(i), flies at the top of the cone enter one of the chambers. After flies have collected in this way for 30 minutes, the cylinder rotates slightly to seal the chamber and an aerosol of metepa is sprayed into the chamber (Fig.1B(ii)). Fifteen seconds later the cylinder rotates again so that the sprayed flies can escape, and so that another compartment is in the collecting position ready to repeat the sequence of operations (Fig.1B(iii)). Flies escaping from the trap appear to fly well clear of it and are rarely seen again on the day of release (A.P.R. House, personal communication). Power to turn the cylinder is provided by a weight, similar in principle to that of a 'cuckoo-clock'. The escapement to regulate the turning, and the valve to produce the spray, are activated by solenoids timed to fire only during daylight hours by an electronic clock with a photosensitive switch. The whole system can be run by eight 1.5V torch batteries and a 100 g aerosol canister that requires replacement every 1–2 months.

Initial work with *G. morsitans* in the laboratory indicated that a high sterility without a high mortality could be induced in males and females by allowing the canister to inject a 5% metepa solution into the treatment chamber for 0.5–1.0s [16]. With *G. morsitans* and *G. pallidipes* in the field the spray durations required to ensure high sterility without high mortality were only 0.1–0.2s

(A.P.R. House, personal communication). The greater sensitivity of field flies may be due to their greater activity in the chamber, during which they could accumulate more droplets.

An important refinement to the device is the provision of a netting lining to each chamber, to ensure that flies do not become stuck on the smear of metapa that accumulates on the perspex after many sprays.

Field trial

Although odour-baited sterilizing traps have not been developed to the point at which they can be used economically for routine control operations, they have been developed sufficiently to justify a small field trial. In September and October 1979, populations of *G. morsitans* and *G. pallidipes* were introduced as pupae to an island of 5 km^2 in Lake Kariba. The populations became well established and in April 1981 a start was made to eradicate them by means of five beta traps baited with carbon dioxide and acetone and fitted with sterilizing devices.

CONCLUSION

There is good reason to suppose that further work could produce odour-baited sterilizing traps that are technologically and economically suitable for tsetse control over large areas. Indeed, such traps are already operating with all the basic requirements supplied in crude forms, which are: an artificial odour that begins to approach the desired level of efficacy; a moderately effective trap; and a sterilizing device which, although perhaps more complex than it need be, does work satisfactorily. Essentially, the problem is to refine the technology that already exists. Undoubtedly, the matter of greatest importance, and the one demanding the highest level of expertise, is the further identification of odour attractants.

But even in its present state, the technology for the small-scale use of odour-baited traps has progressed far enough to provide the field entomologist with convenient and exceedingly powerful tools to achieve many of his research objectives.

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TAGGING OF TSETSE FLIES WITH RADIO- AND STABLE ISOTOPES

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Abstract

TAGGING OF TSETSE FLIES WITH RADIO- AND STABLE ISOTOPES.

Attempts have been made for many years to use radioisotopes for the tagging of tsese flies. Although methods have been worked out which have been practicable, they have not been viable in the field. Tsetse flies are large enough to be painted with colour patterns. Up to now this technique has been simpler and cheaper with small numbers of flies than the handling and measurement of radioactive materials. This was especially true for the administering of the tracer which could only be performed reproducibly through host animals. Today the method of maintaining tsetse species on artificial membranes makes possible the administration of the tracer substances together with the fed blood. Thus labelling of large numbers of flies can be carried out quickly and simply. Consequently internal labelling of tsetse with incorporated isotopes (in contrast to external tagging with dyes) could attract a wider practical interest. Because of radiation safeguards, the application of radionuclides on a large scale would probably not be advisable. They should be used in the field only for special purposes. The marking of very large fly populations, however, can be carried out by the indicator activation method in which stable isotopes are administered to the flies with their blood meal. After a sample has been taken the isotopes are activated by a nuclear reaction and then identified. Although some technical and organizational pre-conditions have to be fulfilled, this method could be a useful alternative to tagging with dyes.

1. INTRODUCTION

The tagging of insects with radioactive and stable isotopes is a very common technique in applied entomology. The large number of investigations published during the last two decades demonstrates a wide variety of tagged species, elements used, methods of administration and purposes of tagging.

Among these references, papers dealing with tsetse flies are extremely rare. There may be some valid reasons for this:

(a) Control programmes, in which the distribution and dispersal of natural and reared populations are studied, only began in the mid 1960s.

(b) As long as tsetse flies could only be fed on host animals, the labelling of large numbers had to be performed through these hosts. Such a procedure, undertaken with radioisotopes, is complicated and dangerous.
(c) The relatively small number of tsetse flies released in current control programmes up to now, made the individual tagging by colours the most advisable and suitable method [1].

In spite of the simplicity, practicability, cheapness and the high information rate of colour tagging, some disadvantages could be significant under certain circumstances. The fly numbers are limited; the label can be washed or rubbed off; chilling and individual handling of flies is necessary; and adults emerging in the field from released pupae cannot be marked. The possibility of feeding tsetse flies on artificial membranes now makes the administering of tracer substances together with the fed blood very easy, perhaps a result of higher interest in isotope techniques. Moreover, there are certain problems which can be solved only by radiation methods, namely the labelling of sperm and its detection in mated females, and the behaviour of individual insects that are not visible.

2. ISOTOPE TAGGING OF TSETSE FLIES

The following review on the tagging of tsetse has been compiled with reference to the most important fields of application, e.g. tagging for release-recapture studies; sperm labelling for investigation of mating capability of released males; tagging for behavioural studies.

2.1. Tagging for release–recapture studies

2.1.1. Radioactive isotopes

Table I summarizes the few available investigations. After injection into the host animal the gamma emitters ^{59}Fe, ^{51}Cr, ^{57}Co or ^{57}Zn were administered orally to *Glossina austeni* Newstead [2–4]. Only ^{59}Fe and ^{65}Zn were obviously suitable for long-term labelling of adults and progeny. The others were excreted within a few days down to a level of radioactivity which was no longer detectable. Azevedo and co-workers [5, 6] labelled *G. morsitans* with ^{32}P, using the same method of administration as the authors mentioned before. A relatively uniform and, in spite of the short physical half-life of the isotope, stable marking of fed females and their progeny was achieved. The last finding was confirmed by our own experiments, where ^{32}P or ^{14}C was injected, and ^{32}P was fed directly to *G. morsitans morsitans* Westwood [7, 8]. The dipping and

spraying of pupae with aqueous solutions of radioactive substances (instead of incorporation by feeding adults) resulted in a low and irregular label [2, 8]. The practical applicability of these alternative techniques is questionable.

In the selection of radionuclides useful for the labelling of tsetse in release projects, some criteria should be kept in mind:

(a) The substances should be physiologically significant and compatible, and should be incorporated.
(b) The biological half-life should be long, so that detection over a long period is possible.
(c) The physical half-life should be as short as possible to reduce the danger of contamination of the environment.
(d) The emitted radiation should be detectable with high sensitivity.
(e) The radiation should have a low energy, capable of being shielded by simple measures.
(f) The price of the material should be low, so that it can be applied in mass rearing experiments.

Of the radioisotopes mentioned, ^{32}P fulfills these criteria best. It is a relevant constituent of important biomolecules so that its chance of incorporation is high. The biological half-life is limited to the relatively short physical half-life. But the latter guarantees fast decay and minimizes the danger to the environment. The beta emission of ^{32}P is, on the one hand, energetic enough to be detected sensitively, even in the living insect, by a great variety of methods, including the very simple and practical techniques of autoradiography using X-ray or Polaroid films [9–11].

On the other hand, ^{32}P can be shielded completely by a few centimetres of paper or plastic material, and is also extremely cheap; 10 mCi, which is about the amount needed to label 10 000 flies, costs DM 175.[1]

The gamma emitter ^{59}Fe was selected because of its affinity to blood constituents [2–4]. The labelling with ^{65}Zn was presumably carried out because of the significance of zinc as a component of many enzymes. However, zinc chloride was proved to be toxic to tsetse at concentrations higher than a few mM. The half-lives of both these radioisotopes are appropriate. They can be identified by any form of gamma detector. Particularly in the field, simple battery-operated monitors can be used. Shielding is not as easy as with ^{32}P, and could produce some problems in the handling of the materials in the laboratory. The price of 10 mCi of ^{59}Fe-sulphate is nearly DM 7000. Zinc-65-chloride is cheaper (about DM 300).

[1] $1\ Ci = 3.70 \times 10^{10}$ Bq.

TABLE I. LABELLING OF TSETSE FLIES WITH RADIOACTIVE AND STABLE ISOTOPES FOR RELEASE/RECAPTURE STUDIES

Species	Labelling substance		Manner of administration	Concentration		Detection method	Ref.
	Radioactive	Stable		Radioactivity	Element		
G. austeni Newstead	^{59}Fe-citrate ascorbate ^{65}Zn-gluconate		Oral via host			NaI(Tl)-gamma detector	[2]
G. austeni Newstead	^{59}Fe-citrate ^{65}Zn-chloride		Oral via host	0.03–0.5 μCi/g host		NaI(Tl)-gamma detector	[3, 4]
G. morsitans	^{32}P-phosphate		Oral via host	1 μCi/g host			[5, 6]
G. morsitans morsitans Westwood	^{32}P-phosphate ^{14}C-leucine		Injection of 1 μl	0.05–1 μCi/μl		Liquid scintill. counting	[7]
G. palpalis palpalis Rob. Desv.	^{32}P-phosphate		Injection of 1 μl Oral via membrane	1 μCi/μl 33 μCi/ml		Anthracene scintill.	[8]
G. morsitans morsitans Westwood		Au-chloride Ir-chloride Dy-chloride Eu-chloride	Oral via membrane		1 mM/l	Indicator activation GeLi-gamma detector	[16]

Species	Labelling substance		Manner of administration	Concentration		Detection method	Ref.
	Radioactive	Stable		Radioactivity	Element		
G. palpalis palpalis Rob. Desv.		Dy-chloride La-chloride Eu-nitrate	Oral via membrane Injection of 1 µl Dipping Spraying		0.03 – 3 mM/l 1 – 100 mM/l 0.1 – 1 M/l 1 M/l	Indicator activation GeLi-gamma detector	[17]
G. palpalis palpalis Rob. Desv. *G. morsitans morsitans* Westwood		La-nitrate Eu-nitrate	Oral via membrane		3 mM/l	Indicator activation GeLi-gamma detector	[18]

TABLE II. NEUTRON ACTIVATION CONSTANTS[a] OF ELEMENTS SUITABLE FOR LABELLING OF TSETSE FLIES

Element	Tracer nuclide	Abundance (%)	Daughter nuclide	Thermal activation cross-section (barn)	Half-life $T_{\frac{1}{2}}$ (h)	Saturation activity As[b] (C/g per TBc/g)	
Lanthanum	$^{139}_{57}La$	99.9	$^{140}_{57}La$	8.5	40.2	10	0.3
Europium	$^{151}_{63}Eu$	47.8	$^{152}_{63}Eu^{m}$	1400	9.3	717	26.5
Dysprosium	$^{164}_{66}Dy$	28.2	$^{165}_{66}Dy$	2100	2.3	592	21.9
Iridium	$^{193}_{77}Ir$	62.6	$^{194}_{77}Ir$	130	19	68	2.5
Gold	$^{197}_{79}Au$	100	$^{198}_{79}Au$	96	64.8	79	2.9

[a] From: BAUMGÄRTNER, F., Table of Neutron Activation Constants, Munich (1967).

[b] Thermal neutron flux density: $10^{13}\ cm^{-2} \cdot s^{-1}$;
Radioactivity after irradiation time t_B: $A = As\left(1 - \exp\left(-0.693\frac{t_B}{T_{\frac{1}{2}}}\right)\right)$.

In addition the isotope ^{14}C was used in distribution studies in the field, not with tsetse, but with other insects [12]. This radioisotope is available as part of many DNA and protein precursors and other essential carbohydrates. Therefore the chance of incorporation of ^{14}C is very high. Its great disadvantage is the long physical half-life of 5730 years, which limits application in tsetse habitats. The range of the emitted beta particles is shorter than 1 mm in any solid material, which makes the radiation protection very simple. For counting, besides such sophisticated methods as liquid scintillation counting or thin-layer autoradiography, the modest autoradiographic technique with Polaroid film can be utilized [11, 13]. The price is high. One of the cheapest significant substances, D-[U-^{14}C] glucose, costs DM 4100 per 10 mCi.

2.1.2. Stable isotopes

The main disadvantage of radioisotopes, namely the potential danger to man and the environment, is excluded if stable isotopes are used for labelling. This is done by means of the indicator activation method [14]. By this technique, the objects under investigation (here the tsetse flies) are marked, externally or by incorporation, with appropriate stable isotopes. Later, only after a sample has been taken, this isotope is made radioactive by a nuclear reaction with thermal neutrons and identified. There is thus no handling of radioactive material during rearing and release, and no radioactive contamination of the environment. Activation and measurement take place in a research reactor station, where special personnel and equipment are available. The physical half-life of the radioisotope does not limit the period of dispersal and observation of the flies. The choice of the indicator is determined by nuclear physical data (activation cross-section and physical half-life), and the same insect parameters as in the case of radioisotopes. The activation cross-section describes the level at which the stable isotope can be made radioactive. The physical half-life should not be too short for practical purposes (at least several hours). From the physical point of view, a great variety of elements could be applicable. However, our own experiments have demonstrated that, for the labelling of tsetse via the blood meal, only a few are useful. Their essential characteristics are listed in Table II. Manganese, which was often used as an indicator in biological field experiments [14, 15], as well as indium and samarium, which all have a high activation cross-section, have been proved toxic at a concentration of 10^{-3} M in the blood fed to *G. morsitans morsitans* Westwood. The chlorides of gold, dysprosium, iridium and europium, at the same concentration, caused little or no mortality in this species. All these elements were detectable following neutron irradiation 20 days after feeding [16]. The amounts of Dy- and La-chloride as well as Eu-nitrate did not reduce survival in *G. palpalis palpalis* Rob. Desv. [17]. But lanthanum gave detectable

labelling for only four days after application to *G. palpalis* and *G. morsitans*. The difference in detectability between europium and lanthanum has a physical reason. The elements have about the same excretion rate. But because of a lower activation cross-section, the detection limit of lanthanum is inferior, and the amount of residual element which is really incorporated and not excreted, cannot be detected as precisely as that of europium [18]. The indicator activation experiments demonstrated that four elements are available for non-radioactive tagging of tsetse flies: gold, iridium, europium and dysprosium. They can be administered singly or together, so that a labelling from various viewpoints is possible.

Another technique that could be useful on a mass rearing scale is the spraying of adults with indicator-containing aerosols [17]. The tsetse populations were maintained in air-conditioned rooms where the required high humidity was produced by atomizers. Therefore we suppose that an additional treatment with an aqueous aerosol would not represent a marked disturbance of the normal environmental conditions. Experiments with lanthanum solutions, up to a concentration of 1M, show a rather homogeneous external labelling of *G. palpalis* which is constant within at least six days.

2.2. Sperm labelling for investigation of mating capability of released males

Only a few papers have dealt with the marking of sperm with radioisotopes and its detection in the females after mating. Nevertheless some authors have reported successful experiments with mosquitoes [19–21], spider mites [22] and pink bollworms [23]. Detectable sperm labelling in F_1 males of *G. morsitans* after oral ^{32}P-intake by pregnant females was reported by Azevedo and co-workers [6]. This statement seems rather inexplicable to me, if the generation cycle of tsetse is considered. Therefore some of our own experiments investigate this question in greater detail. After the injection of ^{32}P-phosphate or ^{14}C-leucine into parental females of *G. morsitans*, labelling of spermatozoa or accessory gland fluid of F_1 males was not detectable [7]. However, *G. palpalis* males fed on ^{32}P-containing blood (activity concentration 33 μCi/ml) transferred 0.15% of their radioactivity to their mates, and 0.034% exclusively to the abdomens [8]. This small but measurable fraction corresponds roughly to the volume percentage of the spermatophore compared with the total volume of a male. Because external contamination of the females could be largely excluded, we concluded that the amount of ^{32}P found in the abdomen was the result of a successful mating. In this experiment males and females were paired for mating individually for seven days, and the females were killed on day 7. Therefore at least some of the females still contained the spermatophores (presumably the females which were most highly radioactive). Normally after one day the spermatophore is expelled when proper insemination

of spermathecae has occurred. But sometimes females retain the spermatophore throughout a longer period. Whereas the spermatophore is mostly of accessory gland secretion [24], the formation of which is delayed until blood feeding begins [25], spermatogenesis in male *Glossina* is completed within the puparium [26, 27]. This means that there is, on the one hand, no possibility to label the sperm by treating the adult males. The abdominal radioactivity of females mated with labelled males must be the result of the mating act and transferred by spermatophore, or parts of the accessory gland secretion which are not transformed to spermatophores. On the other hand, the sperm stored in the spermathecae, if labelled during larval development, is unlikely to be detected because of its small volume (1/15 000 of a total fly), unless there is a preferential accumulation of labelled material in the spermatozoa. No indications for such a process could be found in the literature. Further studies applying radioactive DNA-precursors might help.

2.3. Tagging for behavioural studies

It has been stated above that for dispersal studies — as far as radioisotopes are applied at all — beta emitters are most suitable. Behavioural experiments in natural habitats, where the insects are not readily visible, require isotopes which can be detected over greater distances, and the detection of which is not influenced by plant obstacles. Besides the gamma-irradiating isotopes ^{59}Fe and ^{65}Zn, which are effectively incorporated by tsetse and therefore useful, some additional methods have been experimentally proved. Bois and co-workers [28] tagged *G. palpalis gambiensis* externally with 8 μCi ^{59}Fe each and surveyed the night resting sites of the flies over distances of 1 m and more. Pieces of ^{182}Ta-wire (each containing 85–250 μCi) were stuck to the centre of the thorax of *G. morsitans morsitans* Westwood [29], and the movements of the flies in a field cage were registered over horizontal and vertical displacement up to about 4 m. These experiments illustrate the detection limit for distant gamma sources, which is between 5 and 10 μCi for 1 m. Because, in the administering of radioactive substances by feeding or injection, 1 μCi per fly should not be exceeded, only external tagging of individual flies with higher activities makes useful detection practical over certain distances.

3. CONCLUSION

There are two areas of application where the use of radioisotopes for tsetse tagging is unavoidable. The labelling of accessory gland fluid or sperm, if the latter should be possible, can only be achieved by physiologically essential compounds which contain ^{32}P, ^{14}C or tritium. The tracking of individual

flies in obscure conditions has to be performed by means of gamma emitters such as ^{59}Fe or ^{65}Zn tagged externally or incorporated. In dispersal experiments of large released populations, radioisotopes should and can be dispensed with. Only radiophosphorus seems to be justified from the point of view of radiation protection and costs. If colour tagging is not applicable for any reason, large numbers of tsetse flies can be marked modestly and cheaply by stable indicators administered to the flies during membrane feeding.

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flies in obscure conditions.

USE OF FREEZE-DRIED BLOOD FOR MASS REARING TSETSE FLIES

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Abstract

USE OF FREEZE-DRIED BLOOD FOR MASS REARING TSETSE FLIES.

The aim of this research work was to perfect a membrane feeding technique which does not rely on a regular supply of blood from host animals. Lyophilized bovine blood produced at -40° and 100 Pa in a freeze-drying machine (Edwards Kniese, Marburg, F.R.G.) has been used for feeding a colony of up to 10 000♀♀ *G.p. palpalis* for four years. Approximately 1500 flies could be fed on a feeding surface of 0.22 m^2 with 100 ml of reconstituted blood with the addition of 1×10^{-3} mol ATP. Puparial weight, longevity and fecundity were taken as comparison parameters. With an average of 27 mg from all puparia collected up to day 50, survival rate of 85% and productivity of 2.4 pupae per initial female, there was statistically no significant difference compared with fresh bovine blood used in vitro.

Tsetse flies cover two-thirds of the area of the African continent. Acting as a vector for the trypanosomes they are responsible for human sleeping sickness and for nagana, a pest of high economic impact. To cover the increasing needs of nutrition, control of the trypanosomiasis is necessary. Various measures against this disease have been tried but the most effective, the spraying of insecticides, is a burden to the environment and is increasingly under attack. Under certain conditions a biological control method, such as the sterile insect technique, seems to be the most suitable approach. The limited reproduction capacity of the tsetse fly favours it as a target; however, effective mass rearing of the insect in the laboratory is necessary.

Tsetse flies feed exclusively on blood so the use of host animals was a prerequisite for rearing. The cost and effort involved in the keeping of rabbits, goats or guinea pigs created the demand for an artificial feeding system for the maintenance of the flies. The development of the membrane feeding technique has reached a standard now where it seems to be suitable to be used in Africa for colony rearing

of the tsetse fly. Since the establishment of the silicone membrane, *Glossina m. morsitans* Westwood and *Glossina p. palpalis* have been kept in large numbers on that regimen in Seibersdorf. In that system the fly pierces a specially developed membrane and takes up heated blood. This membrane was originally designed for the use of fresh blood, and the dependency on a blood-donor animal available at regular time intervals remained an unsolved problem. The ability of blood to be stored under refrigeration is very limited, and ongoing denaturation influences its nutritional value the moment it leaves the donor. Therefore we looked for a possibility to store blood in order to overcome the trouble of weekly blood collections without influencing the blood quality. The aim of our research in that direction was to find a way to lyophilize blood which would provide us with the greatest possible independence from blood-donor animals. The required product should be available in large quantities, production should not necessarily take place where the fly colony is being reared, and it should also be suitable for storage and shipment.

The way to reach this goal was found in experiments with haemolysed blood which proved that intact erythrocites are not necessary for tsetse feeding. Bovine blood after collection stored in the refrigerator for some days was no longer suitable for the feeding of *Glossina p. palpalis* compared with fresh blood. It looked as if haemolysis could be the reason for this. But an experiment showed that haemolysed blood was only inferior when the cells had not been separated from the serum, whereas processed blood, which had been separated and where the cells had been washed in saline, could be used. Only the addition of ATP as a feeding stimulus was necessary. Because of these results, we freeze-dried the blood. The defibrinated blood was separated shortly after collection to serum and cell portions and then freeze-dried. During reconstitution the ratio was 1:1. To find out what quantity of ATP is necessary for optimal nutrition with freeze-dried blood, different concentrations were tested. It was found that a higher concentration had to be added as is present in fresh blood. Another test was carried out to increase the proteins in the normal quantity of one blood meal. During reconstitution we took a greater proportion of cells and a smaller of serum. This resulted in a higher osmolarity of the product and reached 310 m/osm. In this experiment it was also tested whether a lower quantity of water during reconstitution could improve nutrition. The results showed that fertility and mean puparial weight were not influenced by the changed osmolarity, but mortality occurred within groups of young flies. As during the first step fertility and puparial weight were less in comparison with fresh blood using freeze-dried blood, we offered the fly more proteins by adding albumin to the serum. Without changing the osmolarity we added 4.5 g bovine albumin to 100 ml of serum, which is the amount normally found in blood. Thereby we were able to increase fertility and puparial weight; however, the experiment showed an atypical trend insofar as normally the first pupae are heavier than the later ones and with albumin it was

vice versa. It can be said, however, that the albumin content of blood has a favourable influence on nutrition of the flies when freeze-dried blood is fed.

Another test was to attempt to use heparinized blood instead of defibrinated blood. During blood collection an amount of 1.5 IU/ml sodium heparin was added to bovine blood. The reconstitution and freeze-drying processes remained unchanged. The results with this product during the experiment showed no significant differences compared with defibrinated blood.

The greatest advantage of freeze-dried blood is its ability to be stored over a long period. At least 6 months storage is possible without any observable loss in quality. We then concentrated on building up a stock colony on freeze-dried blood using bovine, defibrinated blood with the addition of 1×10^{-3} mol ATP. Since July 1977 this colony has been maintained and, except for some technical problems with the freeze-drying machine during the first months, no difficulties have occurred. Productivity and puparial weight could be gradually increased. Later on we changed to another freeze-drying apparatus where we could test different patterns of lyophilization. We were then able to extrude greater proportions of water from the blood which resulted in a better quality product. At that time we switched over from drying cells and serum separately to drying full blood. The proportion of the reconstitution was altered to a cell proportion of 40%. During that period we also tried to lyophilize full heparinized blood, also adding 1×10^{-3} mol ATP. It was proved experimentally that an ATP content of less than 3×10^{-4} mol ATP resulted in reduced longevity of the flies. Groups fed without ATP showed significantly less fertility and puparial weight.

Having found that all results with full freeze-dried blood were better than the previous results, we tried to improve them even further by changing the plasma or serum content of our reconstituted product. Another experiment with bovine albumin was carried out. The results showed a very similar performance, and we came to the conclusion that the latest product could not be improved by addition or change of proportion.

Assuming that we had now found a suitable nutrition for *Glossina p. palpalis*, we compared the performance of flies fed on fresh blood with those fed on lyophilized blood. Both blood types were involved in long-range tests, and neither the defibrinated lyophilized nor the heparinized blood showed significantly different values in longevity, fertility and mean puparial weight compared with fresh blood.

Since January 1979 a colony has been kept on lyophilized, heparinized bovine blood, and in parallel a control was maintained on fresh blood. Soon after the switch in the feeding regimen, the values of the heparinized blood colony reached the values of the fresh blood colony.

Recently, in spring 1980, a colony was initiated on freeze-dried porcine blood and has been kept for comparison. The development of these flies was often hampered by bacterial problems, but during 1981 irradiation of blood with 100 krad has solved this problem.

Looking back to the days when freeze-drying of blood was begun in Seibersdorf in 1975, it must be mentioned that the difficulties encountered at that time were due to the not yet perfected membrane feeding technique and the poor quality of the product dried in an old Leybold Heraeus freeze-drier. Later haemolysed blood guided us to the separation of cells and serum which brought better results. At that time the technique of lyophilization had not reached a standard where full blood could be freeze-dried because the product was not easy to get into suspension after drying. This problem no longer existed with processed blood. After some experience with full blood, which had been haemolysed by adding distilled water, the reconstitution was easier, and the blood powder went into suspension immediately. Thereafter we tried to find a new programme to test lyophilization of full blood and were successful. A minimum dosage of heparin is necessary to prevent coagulation, otherwise the product clots during reconstitution. For the moment freeze-drying seems to be the best way to conserve the highly sensitive portions of blood. For this reason it is absolutely necessary to keep the time between collection of blood and freeze-drying as short as possible. The actual freeze-drying process has to be observed very carefully. The first step is the quick-freezing to obtain optimal crystallization. This can be achieved at temperatures far below the eutectic point. Best results were obtained at $-40°C$ or at a change between $-40°C$ and $-25°C$. After the blood has reached the lowest temperature, it is kept for a minimum of one hour. Then the heating starts at a low vacuum of less than 30 000 Pa, obtaining high convection. Raising the end temperature at a vacuum of less than 100 Pa results in very low residual humidity. The quality control of the product is simply done by feeding tests. A product with a cell portion of 40% needs an equivalent of 4.32 portions of distilled water in weight for the reconstitution and has an osmolarity near 290 ± 5 m osm/kg H_2O, which is equal to fresh blood. The pH with a value of 8.2 is always higher than in fresh blood. Checking the blood quality with a feeding rest it is essential that flies of different age should be offered the product and an equal number of equal flies have to act as control on blood of known quality. Storing the blood in bottles under vacuum or nitrogen saturated atmosphere gives a product which lasts a long time.

We have also observed the considerable impact which lyophilization induces on micro-organisms which cannot be completely avoided during blood collection. As long as the counts of bacteria range in low density, freeze-drying cuts down the surviving germs to a negligible minimum. Complete sterility in cases of heavy infestation could be achieved with gamma irradiation. The latest experiments proved that 100 krad suppressed micro-organisms pathogenic to tsetse flies to a density where they have no influence on the performance of the insects.

Additives to the lyophilized blood seem only then to make sense if the valuable parameters could be improved. This is the case with ATP. Substitutes for the serum portion of the blood have been investigated, but up to now the

results have not been satisfactory; possibly a minimum of serum has to remain and by the addition of albumin some progress can be achieved. We hope that in the near future we shall be able to find a product which no longer needs the addition of ATP by using new freeze-drying techniques. Another step will be to increase the quantity of blood in bottles in order to make shipment of large quantities easier and more economical.

Membrane feeding with freeze-dried blood can now be carried out on any scale at any place independent of blood donors, and thus can be recommended for breeding tsetse colonies in Africa as a basis for the sterile insect technique.

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RECENT ADVANCES IN TSETSE MASS REARING WITH PARTICULAR REFERENCE TO *Glossina palpalis palpalis* (Rob.-Desv.) FED IN VIVO ON GUINEA PIGS

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Abstract

RECENT ADVANCES IN TSETSE MASS REARING WITH PARTICULAR REFERENCE TO *Glossina palpalis palpalis* (Rob.-Desv.) FED IN VIVO ON GUINEA PIGS.

The procedures and techniques for in vivo mass rearing of *Glossina palpalis palpalis* (Rob.-Desv.) developed at the IAEA Seibersdorf Laboratory are described. An account is given of biological parameters and operational components connected with mass rearing to meet the requirements of SIT (Sterile Insect Technique) campaigns. The choice of the optimal fertilization technique and the relevance of puparial weight to general viability and reproductive performance of the progeny is emphasized and illustrated by means of experimental data. Finally, a production model for a nucleus colony containing 100 000 female flies kept in the adjustable stationary phase has been prepared from available information on production figures and dynamics of the *G.p. palpalis* colony maintained since 1974.

1. INTRODUCTION

The Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development, recognizing the magnitude of the tsetse-transmitted trypanosomiasis problem, has for a number of years supported basic research into the possible practical application of the sterile male technique for vector control [1]. As a result, laboratory studies on *Glossina* biology, physiology, rearing, production of sterility by radiation conducted in Seibersdorf [2–6] and in research centres (Lisbon, Bristol, Paris, Antwerp, Amsterdam, Bonn, Hanover, Alberta, York, Toronto, Nairobi, Achimota, Kaduna) participating in the Agency's Co-ordinated Programme, have yielded a wealth of information. Some of the findings have provided guidelines for field testing the SIT concept in Tanzania [7] and Upper Volta [8]. They have also given impetus to the establishment of a third project in Nigeria.

Meanwhile, the rearing of tsetse species of major economic importance has evolved from the maintenance of a few hundred insects per month to meet research requirements [9] to the production of several thousands per day to meet the requirements of a release campaign. In spite of successful propagation of tsetse flies on the adopted mass rearing procedures, it has been recognized [10, 11] that some short-comings and restraints to economical production still exist. Particularly, the low biotic potential of tsetse females and the strict haematophagous character of both male and female flies, make mass rearing a delicate enterprise.

The rapid progress at present being made in the use of artificial feeding methods and the re-orientation towards more appropriate use of animal hosts for feeding the flies, have given rise to a justified optimism for implementation of balanced cost-efficient production schemes.

In vivo methods are here described at present used at the IAEA Seibersdorf Laboratory for rearing the riverine tsetse species, *Glossina palpalis palpalis* (Rob.-Desv.). Some comparative data on previous production procedures are included.

2. MATERIAL AND METHODS

2.1. Colony history

The rearing of *G. p. palpalis* (Nigerian strain) was initiated in July 1974 with 65 female and 50 male flies in the parental generation. The colony originated from field-collected puparia (Abuja Emirate, Niger State) and laboratory-reared puparia sent by the NITR-Kaduna team [12]. Reinforcement occurred on two occasions with 60 and 27 female flies emerging from puparia received from the same source in March 1975 and July 1976. The colony has been maintained on guinea pigs without any further replenishment, and as yet no detectable loss of vigour or reduction in intrinsic reproductive potential has been apparent.

For the period July 1974 through May 1981 the following gross data on colony dynamics have been recorded:

Number of	
Puparia produced	542 468
Puparia retained for breeding	402 180
Female flies emerged	197 007
Male flies emerged	179 675
Overall emergence rate	93.6%
Female ratio at emergence	52.3%

Number of	
Puparia distributed	82 058
Puparia destroyed	32 229
Newly-emerged females distributed	53 668

Over the years, colony expansion was limited at a convenient manageable level of about 4000 female flies with temporary maxima during 1981 of 12 000.

In vivo rearing efforts on *G. p. palpalis* were conducted in two phases. During the first phase, the species' reproductive rhythm, including follicle growth and pregnancy dynamics, was studied under standardized environmental and nutritional conditions. Optimal rearing conditions were defined and general handling and holding procedures, e.g. sexing, mating, separation, feeding, recording, evaluation of performance and quality control, have been optimized gradually. During the second phase, modifications of standard methods have been introduced with major emphasis on some degree of mechanization to reduce the work load and on methods of increasing fly density per holding unit area of production units.

A considerable amount of puparia were used locally in irradiation experiments and several thousands of freshly emerged female flies were used for initiation of membrane-fed colonies. At present, the colony also is a back-up for the Nigerian BICOT Project.

2.2. Insectary conditions

Temperature and relative humidity were controlled by a standard Aerofoil fan, a Lumatic CEB steam-type air humidifier (steam output capacity up to 2.4 kg/h), and calibrated HBC humidistats interconnected to over a central control switchboard.

Over the years, the temperature in the insectary was from 23.5° to 25.0°C and the relative humidity was maintained at 85 ± 5%. Light conditions were kept at 12 h photophase (dimmed light) and 12 h scotophase.

2.3. Host animals for feeding the flies

Most of our work has been done with the guinea pig as host animal. General characteristics regarding its availability, handling, reproduction potential, tolerance-level to bites and interesting haematological features [13] make this animal very useful for experimental and long-term tsetse rearing.

Since the establishment of our *G. p. palpalis* colony, a healthy inbred albino-type guinea pig colony has been kept. Over the years, functional and spatial requirements in the animal house were adapted to cope with tsetse colony expansion. Rational breeding of high-quality guinea pigs was an integral part of the programme.

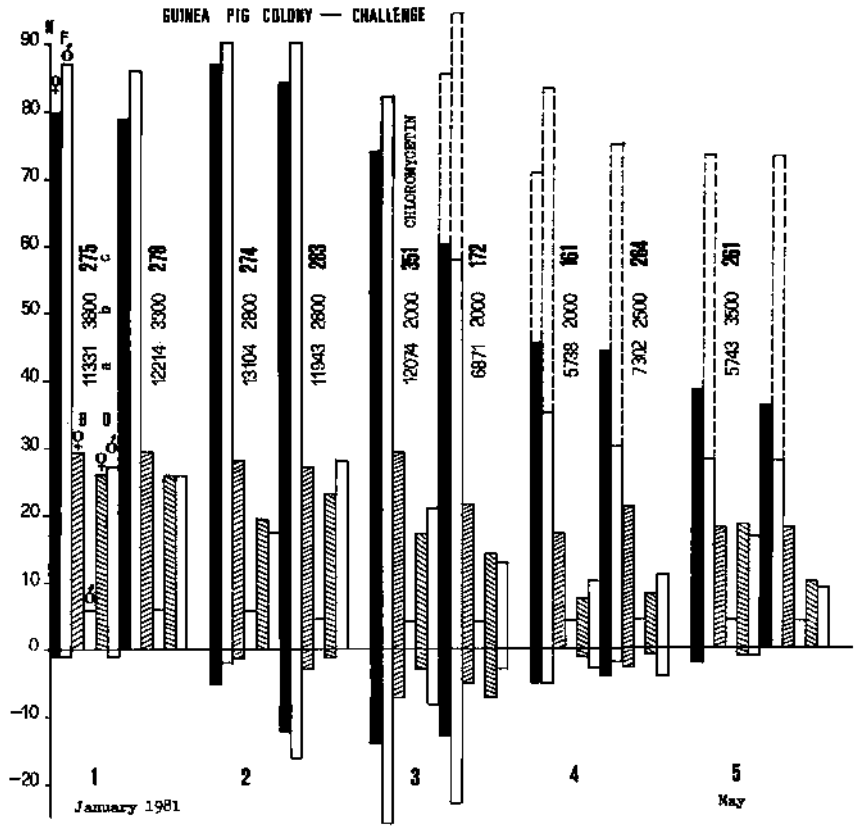

FIG.1. Bar graph presentation of the various categories of guinea pigs kept for in vivo rearing operations during the period January (1) through May (5) 1981.
F = animals used for feeding
B = animals kept as breeders
O = offspring
Bars in dotted line represent new guinea pigs introduced to compensate for the losses from bacterial infection.

During periods of peak numbers in the fly colony, e.g. 12 000 female and 4000 male flies, about 200 full-grown guinea pigs were available. With the standard tsetse feeding regimen being 6 days a week (referred to as 'GP_6'), available hosts were grouped in three lots to be used every second day.

Experience has taught us that it was always best to use animals at least 750 gm in weight. These animals have haematocrit levels of about 45%; plasma and blood volumes were 4 and up to 7.5 ml per 100 g of body weight.

Before being put into daily routine for feeding stock colony flies, each animal received exposure to 60 male flies a day during one week.

Under conditions of adequate food supply – in our case, commercial pelleted diet with regular green vegetable supplement and an efficient level of ascorbic acid added to fresh drinking water – the animals withstood for extended periods of time a challenge of over 250 flies per feeding day without noticeable problems. Mortality among the animals used for tsetse feeding rarely exceeded 2% per month. Some animals have been used continuously twice a week for over a year without showing skin reaction, loss of weight or marked changes in their haematocrit value.

During March 1981, unexpectedly a serious infection went through the guinea pig colony (Fig. 1). On one day, 18 dead animals were found among those used for tsetse feeding. At necropsy, massive internal thoracic haemorrhage was marked and sero-fibrinous lesions were seen. For at least one week mortality continued at a rate of about 5 animals per day, and additional animals were noted which were weak and unstable in their posterior legs. Some had respiratory distress. At the Veterinary Institute (Mödling, Vienna)[1], bacteria isolated from the diseased guinea pigs were identified as *Streptococcus zooepidemicus* and *Pasteurella multicoida.* Immediate chloromycetin therapy (20% injectable) at a rate of 1 mg/100 g bodyweight was recommended and animals relieved from use received at least one injection of antibiotic and two of autogenous vaccine. Mortality continued among the untreated guinea pigs, but some given antibiotic also died. Sick animals in quarantine were given repeated injections when they did not appear to improve. However, recovery, even after therapy, did not occur when the disease was advanced. New animals had to be introduced from the Antwerp University. They were given two vaccine injections and were immediately used in the insect colony. Mid-April, mortality among the old guinea pig stock had ceased and since then animals have been maintained without further problem.

It is noteworthy that examination during the early days of the disease failed to show any evidence of infection in the flies. Furthermore, test-feeding newly emerged *G. p. palpalis* females on animals seven days after their last antibiotic injection, revealed a normal reproductive physiology. Thus, guinea pigs were returned as needed to use in the fly colony at any time after one week following the last injection of chloromycetin. Tsetse colony performance remained unaffected.

The source of the infectious bacteria is unknown. However, the problem started two weeks after a new group of animals to be used for bleeding purposes in membrane feeding tests was purchased locally and placed in the main animal quarter. These new animals showed no abnormal signs until after the disease appeared and became firmly established among the animals already in the room.

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[1] The author wishes to thank R.Gingrich, Head of the Entomology Laboratory, for measures and precautions taken at the onset of the problem. Dr. Mateus of the Mödling Veterinary Institute is greatly acknowledged for diagnosis of reported pathogens and preparation of vaccine.

The episodal problem referred to above serves to illustrate the need for extreme caution when in vivo tsetse rearing is practised. It cannot be over-emphasized that if animals are procured from an outside source, they should be kept in quarantine for at least four weeks in a place remote from operational animal quarters.

2.4. Tsetse rearing procedures and chronology of modifications

During the period of search for the most efficient rearing method, tests have been conducted to determine the specific reproduction rhythm, to define the optimal fertilization technique and fly density per holding cage and to evaluate the relevance of pupal weight to general viability and reproductive performance of the resulting adult fly.

An account is given below of the different steps which led to the final in vivo technique.

1. From the initiation of the colony in July 1974 until June 1975, flies were fed for 10–15 minutes on rabbit ears [14]. For mating, 10 females (2–3-days-old) were caged for 48 h with 12 males (at least 5-days-old) within the standard single oblong PVC cage (18X8X4 cm or about 0.4 l). Sexes were separated by chilling (3°–4°C). Female flies were kept for 120 days following emergence. Their survival and production were checked daily.

2. In July 1975 rabbits were replaced by guinea pigs. Conventional Geigy feeding racks allowing the simultaneous fixation of eight single PVC cages were introduced [15]. Also, the solid sides of the cages were perforated to improve aeration during feeding. Feeding time was reduced to 5 min.

3. At the end of 1975, mated female flies (ratio 10:10 per cage) were regrouped in lots of 20 individuals per perforated double-sized (16X8X9 cm or about 0.8 l) oblong PVC cage (referred to as DS cage). Feeding regimen remained unchanged.

4. During 1976, 1977 and 1978 newly-emerged females were grouped as usual at 10 per single cage until the day of mating (day 2 post-emergence). However, for mating only four males were added to 10 females. Instead of separating the sexes after 48 hours, mates were kept together until the females reached about the time of their first ovulation, i.e. day 8–9 post-emergence. Females were then regrouped at 30 or 40 individuals per DS cage. Holding time was reduced first to 95 days and afterwards to 80 days. In addition to the DS cage, tests were made with cylindrical aluminium

FIG.2. Tsetse fly holding trolley: 36 containers (200 females and 80 male flies per container).

cages (50 cm high and 20 cm in diameter) holding up to 300 mated females. Furthermore, a GP_5 feeding regimen and increased fly density (50, 60 and 70 mated females per DS cage) were tested.

5. Since mid-1979 an alternative in vivo rearing system has been adopted. New types of feeding racks, feeding trolleys and holding trolleys were introduced A cheap and handy 10-l plastic-container has been modified into a holding cage. For the rearing procedure as now finalized, the 10-l containers are

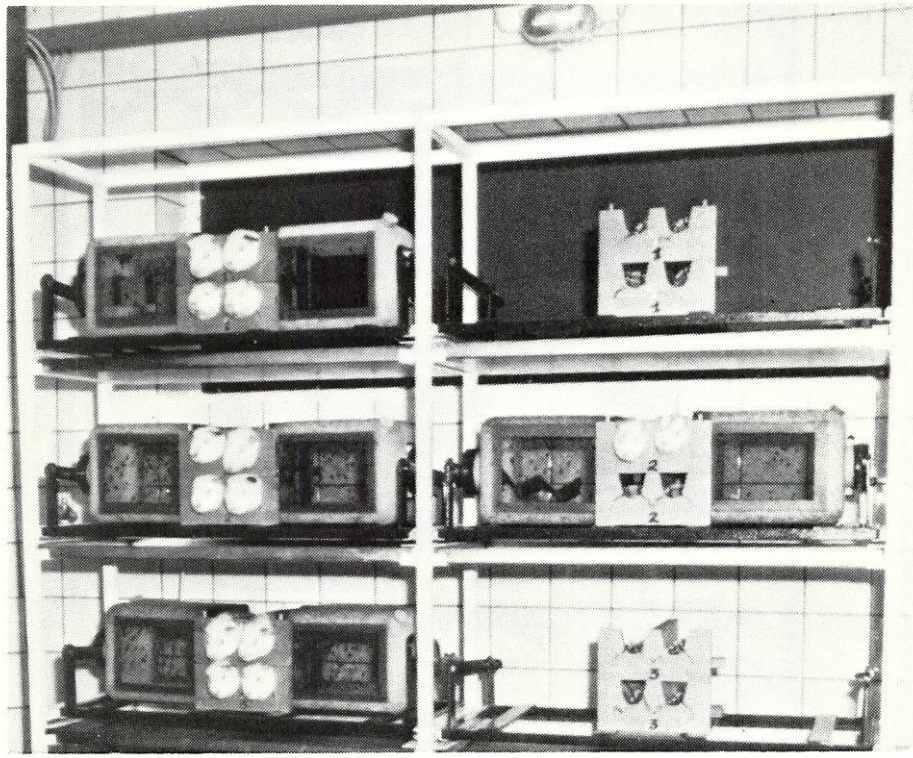

FIG.3. Mobile feeding unit accomodating 32 guinea pigs (eight pairs of piled feeding racks).

filled with 200 females, transferred immediately during chilling at emergence, and 60–60 males (at least 5-days-old), added when the females are 2-days-old. Sexes remain together and each container is taken out of production after 80 days. The holding trolley (120X60 cm and 190 cm high) (Fig. 2) contains three tiers with 12 containers each hanging above a larviposition tray. The trolley has a storage capacity of 7200 female and 2160–2880 male flies. In conjunction with the holding trolley, a mobile feeding unit (180X50 cm and 190 cm high) (Fig. 3) has been designed to accomodate eight removable pairs of piled racks into which up to 32 guinea pigs can be inserted. A droppings tray is mounted underneath each set of four animals. During feeding, the netting-side at the bottom of the containers is in close contact with a flank of two animals (Fig. 4). With a full load of 32 animals and 16 holding containers, the system described offers the potential of feeding at the same time 3200 female and 920–1280 male flies per rotation which lasts on the average 15–20 min. An additional advantage is the reduced handling since all flies can be fed in the holding room.

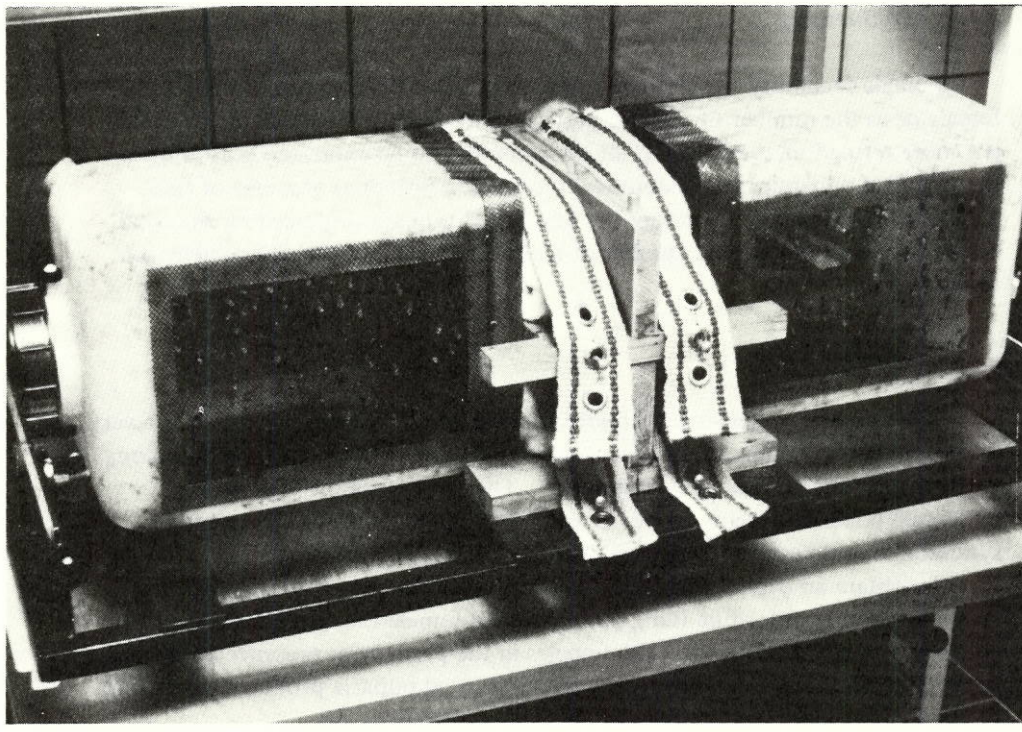

FIG.4. Close-up of one feeding unit. The netting-side at the bottom of each fly container is in close contact with a flank of two animals.

2.5. Tsetse performance parameters

When standard rearing procedures were used, e.g. individual tubes or cage types with a limited number of flies, a daily check was made and the performance was measured in terms of survival, puparial production and weight of puparia produced.

Since the 10-l containers have been introduced, puparia have been collected routinely from the larviposition trays every day, but the production units checked for mortality only at 5-day intervals.

2.5.1. Longevity of females

Longevity within production units was characterized by the percentage of females surviving until the day of expected first larviposition, i.e. day 18 following emergence, and by the survival rate during the mature female period, i.e. from day 18 through the end of the holding period.

experimental or theoretical number of mature female days through which each female lived.

2.5.3. Puparial weight

The puparial weight together with survival and larviposition rhythm is a very important factor among the quality control parameters to evaluate tsetse colony performance under laboratory conditions. The production of puparia and individual puparial weights reflect to what extent the female flies succeed in synchronizing their reproductive cycle with the feeding rhythm [16].

To obtain an overall picture of the quality of puparia produced with the standard holding procedure (GP_6 and 30 mated female flies per DS cage) and the alternative method (GP_6 with 200 females in the permanent presence of 60 males in the 10-l container), daily records were kept of all puparia produced according to consecutive larviposition days between day 16 and day 85.

At a later stage of our rearing programme, the mechanical pupal calibration device [17], which has been recommended and used as a component of standardized quality control procedures in fruit fly mass-rearing plants [18], has been introduced for routine screening of size/weight of tsetse stock colony puparia. In this system, puparia are moved between two sloping rotating steel cylinders with increasing interspace.

In order to find the most suitable interspace, several thousand puparia (collected within the first 24 h of larviposition) have been processed through the machine and subsequently weighed individually. Finally, five distinct size classes of puparia have been selected and their corresponding mean weights defined. Representative samples from randomly collected stock colony puparia belonging to each of the five classes were incubated under standard conditions and their emergence rate and sex ratio were checked.

In tests designed to determine the relevance of puparial weight for female fecundity, female flies recovered from puparia belonging to a particular weight class were mated in standard DS cages (ratio 40 females : 20 males) to randomly collected stock colony males. The fecundity was recorded for 55 days following emergence.

Additional experiments were conducted to examine the reliability of the pupal sorting machine for assessment of the actual number of puparia produced

daily within the stock colony. Puparia produced during consecutive days were collected daily and processed through the pupal sorting machine. The total weight of all puparia belonging to a particular size/weight class was determined and divided by the class mean weight defined during earlier calibration tests. The value obtained was compared with the value resulting from exact counting.

Tests for screening the fecundity of females emerged from puparia in distinct weight classes were repeated. However, this time the alternative maintenance procedure (10-l container with 200 females and 80 males) was adopted. Female and male flies that emerged belonging to the same weight class were mated. Productivity during a 50-day period was followed and all females were dissected at the end of the experiment.

3. RESULTS: BIOLOGICAL PARAMETERS RELATED TO EXPERIMENTAL AND COLONY FLIES

3.1. Reproduction rhythm of individual females

Figure 5 gives information on the reproductive performance of a batch of 30 female flies, mated two days post-emergence, after 24 h separated from their seven-day-old mates and kept in individual fly cages. Their pupal production under standard rearing conditions (24.5° ± 0.5°C; 85 ± 5% R.H. and GP_6) was recorded during a test period of 55 days.

It is clear that there are variability in timing of the first larviposition – ranging from day 16 to day 23 post-emergence – and slight differences in duration of consecutive reproductive cycles – ranging from 8 to 11 days with a mean of 9.36 ± 0.25 days.

Also illustrated are sources of possible loss in overall fecundity which generally are: early mortality, lack of insemination, abortion and mortality in an advanced stage of pregnancy.

During the 55 days test period, the mean number of puparia produced per initial female was 3.96 (N = 119) and the mean puparial weight exceeded 32 ± 3.02 mg.

This pilot test clearly demonstrates the very low potential of tsetse females even under the most optimal holding conditions.

Complementary experimental work on *G.p. palpalis* [19] has revealed that the size of the primary egg follicle in a newly emerged female can vary considerably depending upon the size of the fly and correspondingly upon its earlier puparial weight. It has also been shown that females that emerge from puparia with sub-optimal weights, or females that are reluctant to feed during the first few days of their life, display a marked delay in egg maturation and ovulation. Such females, instead of ovulating their first egg by day 9 or 10, usually have not

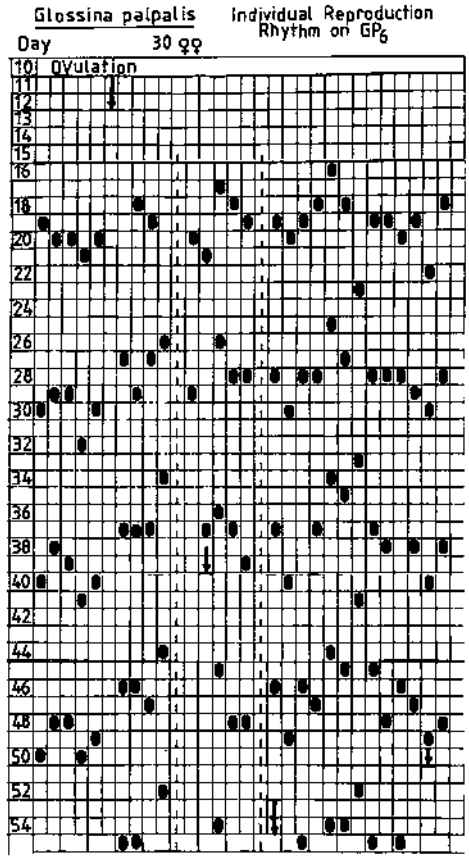

FIG.5. Performance (survival and pupal production) of female Glossina palpalis palpalis *kept for 55 days following emergence. Black ovals represent individual puparia resulting from larviposition at indicated days following emergence.*

ovulated by day 12 or 13, and their first larva is subsequently also retarded. This explains some of the data illustrated in Fig. 5.

3.2. Optimal fertilization technique

During mating tests with *G. p. palpalis* it was found that male flies are willing to remate and are capable of producing three to seven sperm-filled spermatophores during the period five to nine days post-emergence. This indicates, as found for *G. m. morsitans* [20], that *G. p. palpalis* males can be effectively reused at least three to four times. Thus, instead of using the conventionally adopted 1:1 ratio [9], the female/male ratio can be optimized.

TABLE I. STATUS OF THE IN VIVO *Glossina palpalis palpalis* COLONY AT THE IAEA SEIBERSDORF LABORATORY DURING 1980 AND 1981

	Females in stock[a]	Mean percentage daily mortality	Viable pupae produced	Female flies emerged	Male flies emerged	Puape distributed	Newly emerged ♀♀ distributed
1980							
January	4 593	1.05	9 234	4 855	3 699	6 138	368
February	6 871	0.98	11 211	1 289	890	4 949	20
March	4 613	1.15	8 020	3 500	3 177	2 459	0
April	4 502	1.22	8 045	2 055	1 398	600	247
May	4 535	1.09	9 855	4 515	3 525	850	161
June	5 332	0.95	11 575	3 938	2 773	200	541
July	4 415	1.34	10 762	5 619	3 955	0	2 003
August	6 789	0.88	14 060	5 491	4 081	1 957	113
September	9 862	0.92	18 906	6 906	5 670	4 298	1 533
October	10 626	0.85	20 980	1 594	1 271	15 226	1 400
November	7 077	1.55	12 848	6 179	4 720	0	1 148
December	10 340	1.31	18 824	7 656	5 353	0	781
1981							
January	12 214	1.06	24 994	9 552	7 047	1 100	3 255
February	11 943	1.25	21 971	11 170	8 385	3 491	5 505
March	6 871	1.07	19 862	10 548	8 189	2 621	7 883
April	8 622	1.29	15 489	8 856	6 370	1 396	3 380
May	7 750	0.78	12 440	7 185	5 653	0	3 040

[a] By end of month.

Among our stock colony, female/male mating combinations of 10:4 (in single PVC cages; separation after five days), 20:8 (in DS cages; separation after five days) and 200:80 (in 10-l container; without separation) yielded similar overall insemination as 10:10 combinations (in single PVC cages; separation after 48 h), namely at least 95%.

In large-scale rearing operations designed for producing excess males for radiation sterilization and release in the field, a reduced male ratio for mating and multiple use of males is highly desirable because:

(a) male development in the puparium lasts on the average two days longer than that of the females;
(b) males are not fully potent before day 5 – their accessory gland functions are to be activated by consecutive blood-meals [21] – whereas females become highly receptive shortly after their first blood-meal;
(c) a reduced male ratio for mating purpose saves rearing space, simplifies handling and reduces the challenge to the host animals.

3.3. Stock colony dynamics

Table I gives gross data on the numerical evolution of the stock colony since the alternative rearing system with the 10-l containers has been used.

The fluctuations observed in mean percentage daily mortality (0.78–1.55 for the total colony) were mainly due to changes in age composition of the colony which were in turn caused by irregular outputs, both quantitatively and temporarily, of newly emerged female flies and puparia. It might be assumed that for the rearing method adopted, mortality consistently fluctuates around 1.0% for a colony in which pre-productive females, younger than 18 days, represent 25–30%, and producing females, up to 50- and 80-days-old, represent about 50% and 20–25% respectively. For reasons indicated above, at some periods during 1980 and 1981, the age composition of our colony shifted to a higher proportion of older females.

3.4. Comparative data on female fly performance

Information collected on survival and productivity from stock colony units kept under various holding procedures is given below.

3.4.1. Production units based on mating ratio of 10:10 (48 h) and 10 mated females kept in single PVC cage (0.4-l)

Number of females	5000
Average percentage early mortality, i.e. among females younger than 18 days	1.50
Average percentage daily mortality among mature females, i.e. period day 18 to day 80	0.65
Survival rate (%) calculated relative to the theoretical maximum number of mature female days	
By day 50	93.5
day 80	86.0
Fecundity, expressed as mean number of puparia per initial female per day for a recording period of 80 days	0.058

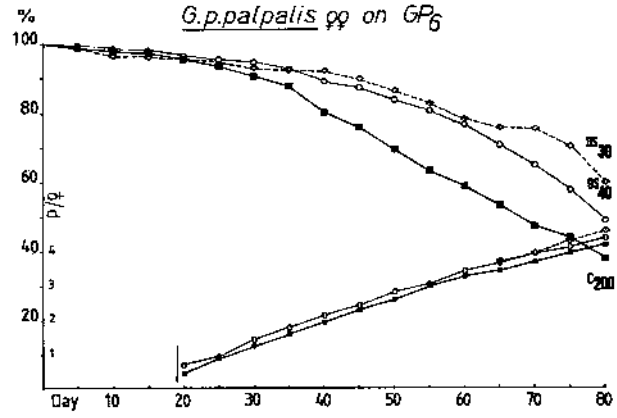

FIG. 6. Comparison of the performance (survival and pupal production) of female Glossina palpalis palpalis *kept in DS cages (0.8 litre with 30 or 40 mated female flies) and container cages (10 litres with 200 females and 60–80 males).*

3.4.2. Production units based on mating ratio 10:10 (48 h) and 20 mated females kept in DS cage (0.8 l)

Number of females	5000
Average percentage early mortality among immature females	2.20
Average percentage daily mortality among mature females	0.74
Survival rate (%) during mature period	
By day 50	92.1
day 80	83.7
Fecundity, expressed as mean number of puparia per initial female per day for a recording period of 80 days	0.054

3.4.3. Production units based on mating ratio 20:8 (5 days) and 30 or 40 mated females kept in DS cage, and on use of 10-l container with 200 females and 60 or 80 males kept without separation

In Fig. 6 and Table II, a comprehensive comparison is made of the overall performance of control units kept by the standard (DS) and the alternative (container) rearing method.

TABLE II. COMPARISON OF PERFORMANCE OF *Glossina palpalis palpalis* IN DS CAGES AND IN CONTAINERS
Survival and fecundity during consecutive cycles expressed in terms of mature female days

	DS_{30} (N = 180 separated females)					10-litre container (N = 200 females × 60 males)				
	Survival in mature female days				Fecundity	Survival in mature female days				Fecundity
	Theoretical maximum	Experimental value		Daily mortality	Puparia/ female	Theoretical maximum	Experimental value		Daily mortality	Puparia/ female
Day 1–17	3 060	3 000	98.0%	0.35%	0.14	3 400	3 311	97.9%	0.17%	0.25
18–24	1 260	1 210	96.0	0.08	0.77	1 400	1 341	95.7	0.22	0.73
25–34	1 800	1 714	95.2	0.23	1.55	2 000	1 846	92.3	0.54	1.57
35–44	1 800	1 658	92.1	0.42	2.24	2 000	1 715	85.7	1.10	2.45
45–54	1 800	1 567	87.0	0.83	2.93	2 000	1 521	76.0	2.10	3.15
55–64	1 800	1 419	78.8	0.77	3.55	2 000	1 253	62.6	2.55	3.66
65–74	1 800	1 332	74.0	0.75	4.22	2 000	905	45.0	2.50	4.01
75–84	1 800	1 115	61.9	2.50	4.85	2 000	742	37.1	2.42	4.32
	12 060	10 015	83.04	0.84	4.85	13 400	9 323	69.5	1.71	4.32
	Number of puparia produced				873					864
	Puparia relative to experimental value of mature female days				0.087					0.092
	Puparia relative to theoretical maximum of mature female days				0.072					0.064

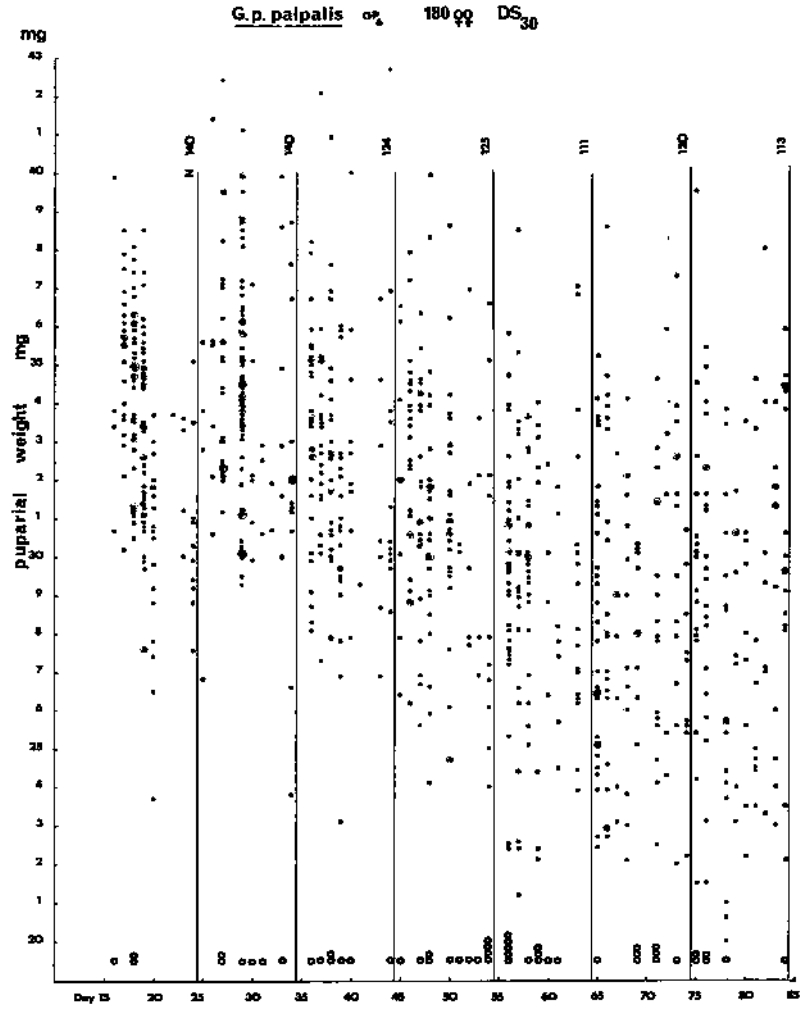

FIG. 7. Weight of individual puparia produced at indicated times following emergence. Data are pooled from six DS cages (0.8 litre) containing 30 mated female Glossina palpalis palpalis.
• = *individual puparial weight*
● = *3–5 puparia with identical weight*
○ = *unviable puparia (undersized and anormal pupation).*

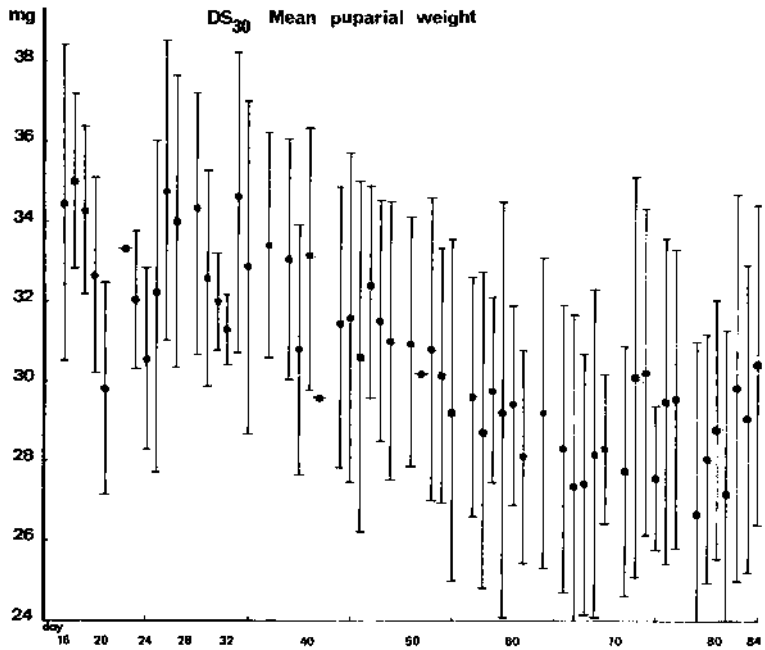

FIG.8. Fluctuations in mean puparial weight in the DS_{30} holding system: ● = 3–5 puparia with identical weight.

Production data for units in which female flies are grouped in lots of 30–40 individuals per cage and kept for 80 days on a 6-days-per-week feeding regimen, can be considered as being close to the species' breeding potential under insectary conditions. There was less than 5% overall early mortality during the period extending from the day of first chilling at emergence through the mating period and a second chilling for separation until day 18 post-emergence; there was less than 1.0% mean daily mortality among potential producing females and at least 50% actual survival by day 80. The number of puparia produced per day for any group of 1000 mated females ranged from 52 to 56. This represented a daily average surplus over maintenance needs of 29 puparia per 1000 stock colony females.

Information collected during 1980 and 1981 on 140 production units, each containing 200 females and 80 males and kept for 80 days, showed consistent performance. For each batch of 1000 emerged females, about 4000 viable puparia were obtained (the number of puparia yielded per initial female ranged from 3.87 to 4.35).

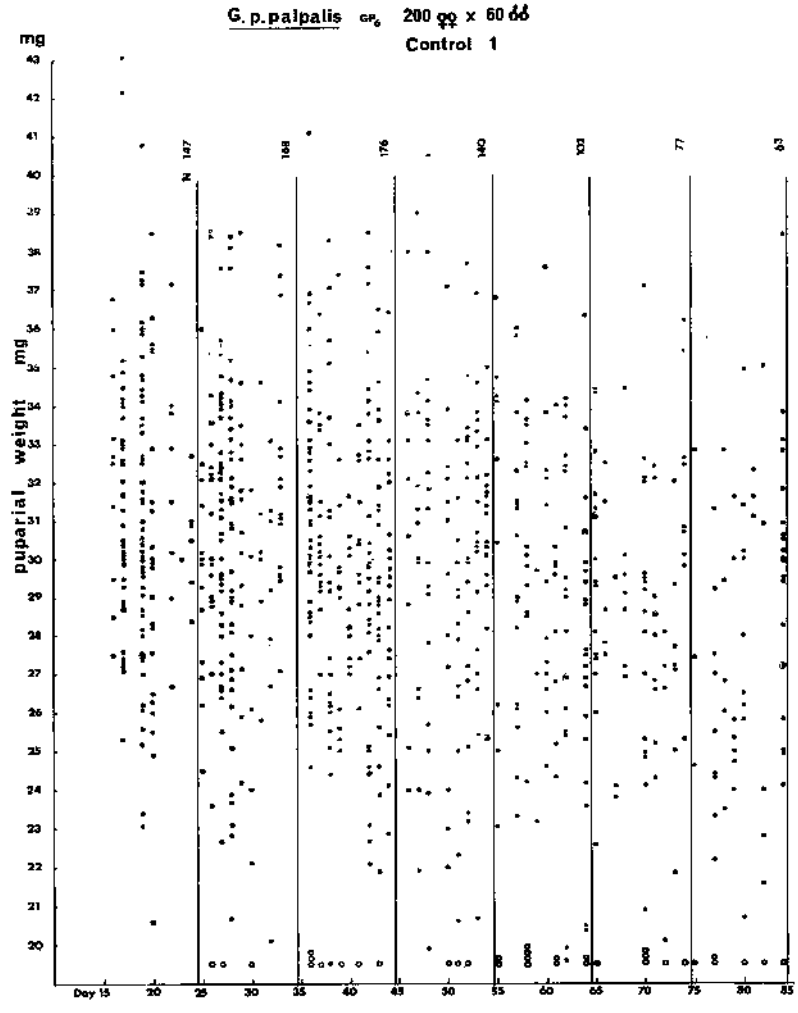

FIG.9. Weight of individual puparia produced at indicated times following emergence. Data are from a control unit in which 200 female and 60 male flies were kept in the 10-litre container.

• = individual puparial weight

○ = unviable puparia (undersized and anormal pupation).

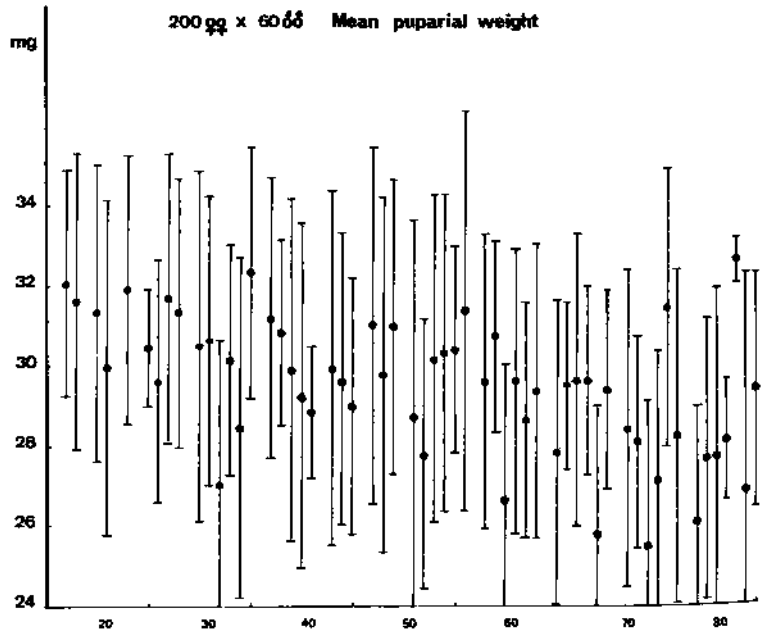

FIG.10. Fluctuations in mean puparial weight in the container holding system: ● = 3–5 puparia with identical weight.

The fecundity factor obtained with the alternative rearing system was about 10% lower than the one with the DS_{30} system. However, this relatively slight difference, which was mainly due to a more pronounced daily mortality among flies older than 40 days (Fig. 5), is outweighed by the benefit gained because of reduced handling (no second chilling for separation after mating), lower space and labour requirements.

Thus, the alternative rearing method may be considered as very efficient and suitable for large-scale rearing of *G. p. palpalis.*

3.5. Puparial weight and its relevance to general viability and reproductive performance of the progeny

From previous experience (unpublished data), it is known that if flies are kept singly the puparial weight remains fairly constant (i.e. above 30 mg) during the first 6–8 reproductive cycles. Among older flies the puparial weight falls towards the end of the reproductive life. It has been observed that wing damage in older females fed on guinea pigs leads to a breakdown in the normal feeding pattern. Such females became infrequent feeders and produced smaller pupae.

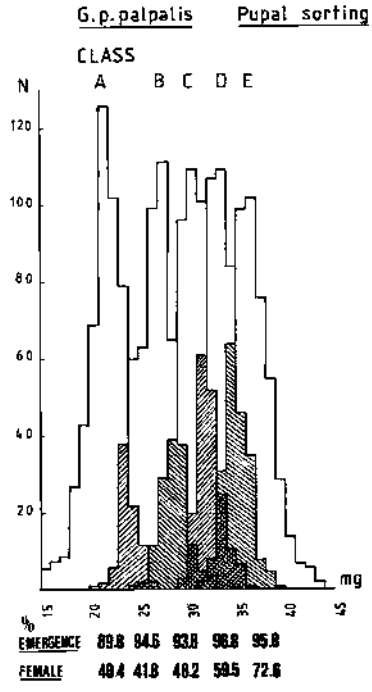

FIG.11. Frequency distribution curves for individual Glossina palpalis palpalis *puparia recovered in five distinct size classes (500 puparia per class) during calibration tests of the pupal sorting machine.*

Figures 7—10 show the individual weight and mean weight of puparia produced on consecutive larviposition days and within each reproductive cycle by female flies in the DS and container holding system.

From these figures it can be concluded that the DS_{30}-females produced somewhat heavier puparia in their earliest reproductive cycle than the container females. It is worth noting that in spite of an inherent higher fly activity in the container, the spectrum of puparial weight distribution within subsequent cycles did not differ markedly from the standard picture. The only significant difference was an increase in percentage of puparia with a weight less than 24 mg (up to 8% in the container versus about 4% in the DS cage).

Thus, there was no apparent crowding effect interfering with the container flies' feeding behaviour.

3.5.1. Calibration of pupal sorting machine

Figure 11 shows the frequency distribution curves for individual weights of 500 puparia recovered in five distinct size classes (A, B, C, D, E) after processing

TABLE III. PUPAL SORTING: CALIBRATED MEAN WEIGHTS OF *Glossina palpalis palpalis* PUPARIA FROM IN VIVO COLONY IN FIVE CLASSES

Emergence rate and female fly ratio

Class[a]	Mean puparial weight (mg ± S.D.) Five replicates (100 puparia each)					Total	Emergence		
	1	2	3	4	5	N = 500	% Total	% ♀♀	% (♀♀)[a]
A	21.12 ± 2.10	20.43 ± 2.08	20.76 ± 1.92	21.02 ± 2.03	21.56 ± 1.14	20.98 ± 1.89	89.8	40.4	42.4
B	26.49 ± 1.89	26.16 ± 1.89	26.29 ± 2.13	25.98 ± 2.07	25.92 ± 1.96	26.17 ± 1.99	94.6	41.8	42.8
C	30.43 ± 1.65	29.90 ± 1.75	30.16 ± 2.05	29.68 ± 1.82	29.64 ± 1.92	29.96 ± 1.84	93.8	48.2	49.0
D	33.39 ± 1.84	33.09 ± 1.79	32.64 ± 1.90	32.77 ± 1.56	32.57 ± 1.79	32.89 ± 1.78	96.8	59.5	60.4
E	35.73 ± 1.67	36.18 ± 2.18	36.01 ± 2.36	36.13 ± 1.89	36.08 ± 1.90	36.03 ± 2.00	95.8	72.6	74.8

[a] Total female percentage, including females recovered from non-emerged puparia;
Puparia with lysed content in each weight class: A (5.8%) B (3.8%) C (2.6%) D (91.8%) E (1.0%).

through the pupal sorting device. Table III gives the mean weights for five replicate groups of 100 puparia each and the total mean weight (± S.D.) for each class. Additional information is given on the overall emergence rate and the female ratio within the various classes.

The data indicate some qualitative differences between puparia of the various weight classes. Class A puparia had an inferior emergence rate, but there were no significant differences in emergence rate for puparia from the other classes. However, there was an interesting trend in the sex ratio with more females emerging as the pupal weight increased.

3.5.2. Fecundity of female flies related to puparial weight

Information collected during tests with DS_{40} cages is summarized in Table IV. The performance of females in class A, in spite of adequate insemination, was clearly inferior to females in other classes. This was mainly due to slightly higher early mortality and a markedly lower survival rate during the mature period. For class B through class E females, the break-even point of 2.5 puparia, i.e. where females start producing distributable profit, was reached after a fairly consistent time period following emergence, i.e. around day 40. Fecundity values obtained for these females after 55 days were very close to the species' theoretical biotic potential under the prevailing insectary conditions. Values above four puparia per initial female or above 0.105 puparia per mature female day might be considered as exceptional, and can only be explained by a very early first ovulation and/or a shorter duration of the reproductive cycle than the expected 9.5 days. It is also noteworthy that the mean weight of puparia produced by females from class A was significantly lower than for other classes.

Information collected during tests with 10-l containers and mates belonging to the same weight class is summarized in Table V.

The following conclusions can be drawn:

1. There was no significant difference in the insemination rate of females within the various weight classes. Thus, males emerging from the lightest puparia (class mean weight 20.9 mg ± 1.89) showed similar mating efficiency (95%) to those from heavier weight classes (95–98%).

2. Early mortality during the immature period among females of class A and even class B was markedly higher than in the other classes, which might indicate some intrinsic weakness.

3. In spite of similar survival rates during the mature period, the break-even point of 2.5 puparia/initial female was not reached by females of class A and B by the end of the 50-day test period. Some of the females apparently

TABLE IV. PERFORMANCE OF *Glossina palpalis palpalis* FEMALES EMERGED FROM PUPARIA IN DISTINCT WEIGHT CLASSES

Mating procedure: 40 females (particular class) × 20 males (randomly sampled) (5 days)
Holding procedure: DS_{40} kept for 55 days on GP_6 feeding regimen

Batch	Original puparial weight (mg)	No. of females	Insemination rate (%)	Early mortality	Survival rate day 18–55	No. of puparia produced (no unviable)		Puparia/ initial ♀ by day 20	Puparia/ initial ♀ by day 55	Day of break-even point	Puparia/mature female day (experimental value)	Puparia/mature female day (theoretical maximum)	Mean puparial weight (mg)	S.D.	S.E.
$A_{(1)}$	21.1	40	100	5.00	85.2	113	(2)	0.02	2.82	49	0.087	0.074	28.2	3.42	0.30
$A_{(2)}$	21.5	40	93.7	7.50	75.3	96	(2)	0.12	2.40	56	0.083	0.063	27.6	3.69	0.37
$B_{(1)}$	26.4	40	97.5	5.00	92.3	157	(1)	0.82	3.92	40	0.111	0.103	30.0	3.07	0.25
$B_{(2)}$	25.9	40	100	2.50	91.4	172	(2)	0.95	4.30	37	0.123	0.113	30.6	3.63	0.27
$C_{(1)}$	30.4	40	97.5	5.00	82.8	141	(5)	0.67	3.53	42	0.103	0.092	31.5	4.13	0.34
$C_{(2)}$	29.6	36	97.2	5.60	93.5	150	(2)	0.88	4.16	41	0.117	0.109	32.6	3.66	0.29
$D_{(1)}$	33.3	40	100	2.50	86.5	150	(1)	0.77	3.75	40	0.114	0.098	32.4	3.78	0.30
$D_{(2)}$	32.5	40	97.5	5.00	91.3	158	(1)	0.85	3.95	40	0.113	0.104	33.5	3.71	0.30
$E_{(1)}$	35.7	40	100	0.00	93.3	154	(4)	0.82	3.85	41	0.108	0.101	34.0	3.66	0.29
$E_{(2)}$	36.0	40	100	7.50	88.0	145	(5)	0.75	3.63	41	0.108	0.095	33.5	3.71	0.30

had difficulties in synchronizing their pattern of blood intake with their reproductive rhythm. Furthermore, the differences in number of puparia/initial female by day 20 (less than 0.5 for females from classes A and B) were indicative of a delay in timing of egg maturation and first larviposition.

4. The frequency distribution of weights for F_1 puparia, determined by the pupal sorting machine, showed increasing representation of heavier puparia from parent production units A (class C + D + E F_1 puparia represented only 18% of the total) through unit E (class C + D + E F_1 puparia represented over 70% of the total).

3.6. Use of pupal sorting machine as counting device

The data shown in Table VI indicate that differences found between the exact number (as determined by manual counting) and the calculated value (as determined by the biomass divided by the class mean weight) were very small. The total error was less than 3%, and less than 2% for the puparia in the midclasses, which had the highest frequency values.

These findings are interesting from a practical viewpoint because it was shown that the use of the pupal sorting device offers a rapid and accurate method for assessing the number and quality of the pupal production in a mass-rearing operation.

4. PRODUCTION MODEL

On the basis of experience with the in vivo technique at present in use, estimates of the requirements to maintain a colony of 100 000 female *G. p. palpalis* have been made.

It is assumed that such a colony is kept in the adjustable stationary phase, and that the females are kept for 80 days following emergence. For both logistic and safety reasons it is also recommended to keep the entire breeding stock in two separate nucleus colonies each with about 50 000 female flies and about 15 000 stud males for mating purposes.

4.1. Operational parameters

Estimations for each nucleus colony kept in an insectary with floor dimensions 6 X 5 m (30 m^2 holding area):

(a) Lumatic air humidifiers CEB II (2)
(b) Airconditioners (2)
(c) Humidistats (4)

TABLE V. PERFORMANCE OF *Glossina palpalis palpalis* FEMALES EMERGED FROM PUPARIA IN DISTINCT WEIGHT CLASSES AND MATED TO MALES EMERGED FROM PUPARIA IN SAME CLASSES

Holding procedure: 10-litre container (200 X 80) kept for 50 days on GP_6 feeding regimen

Origin of parent material	No. of females	Insemination rate (%)	Early mortality	Survival rate day 18–50	No. of viable puparia	Puparia/initial ♀ by day 20	Puparia/initial ♀ by day 50	Puparia/mature female day (experimental value)	Puparia/mature female day (theoretical maximum)	Frequency distribution of F_1 puparia				
										Classes				
										A	B	C	D	E
A X A	200	95.0	6.0	87.7	482	0.39	2.41	0.083	0.073	27.59	54.30	16.33	1.54	0.22
B X B	200	96.0	5.0	84.3	469	0.43	2.34	0.084	0.071	13.46	42.16	33.55	8.83	1.98
C X C	200	98.0	2.5	90.9	576	0.65	2.88	0.095	0.087	6.69	35.93	40.49	15.20	1.71
D X D	200	98.0	2.5	80.9	525	0.69	2.62	0.098	0.079	6.28	31.86	39.83	18.02	3.98
E X E	200	95.0	2.0	81.39	582	0.77	2.91	0.108	0.088	5.10	25.47	39.27	22.35	8.81

TABLE VI. RELIABILITY TEST OF THE PUPAL SORTING MACHINE FOR ASSESSMENT OF THE ACTUAL NUMBER OF PUPARIA PRODUCED IN THE *Glossina palpalis palpalis* STOCK COLONY

Frequency distribution of puparia (produced during 12-day period daily sampling)

Class	A	B	C	D	E	
Class mean (mg)	(20.98)	(26.17)	(29.96)	(32.89)	(36.03)	
Total weight of puparia recovered in class (g)	15.287	62.475	82.810	44.731	13.860	
Calculated number of puparia	728.6	2387.1	2763.3	1359.7	384.6	7623.3
Exact number of puparia (counted)	766	2471	2815	1375	394	7821
Deviation (%) between calculated and real number	− 5.13	− 3.51	− 1.87	− 1.12	− 2.44	− 2.59
Frequency value (%) based on calculated number	9.55	31.31	35.24	17.83	5.04	
Frequency value (%) based on counted number	9.79	31.59	35.99	17.58	5.03	

(d) Holding trolleys (8)
Each trolley accommodates 36 holding containers (10-1 and 200 females X 80 males)

(e) Feeding trolleys (4)
Each trolley accomodates 8 pairs of double guinea pig racks

(f) Guinea pig racks (32 double sets)

(g) Holding containers (288)
With an average stockage rate of 75% an additional 40 containers might be required

(h) Wall shelves for accomodation of cages with stud males and puparia

(i) PVC cages (0.4-l) for stud males (500)

(j) Guinea pigs

To keep the challenge per animal below 250 flies/feeding (twice a week), 256 guinea pigs should be available per feeding day. The GP_6 regimen with use of the same lot of animals every second day thus requires 768 full-grown guinea pigs. A contingency of at least 5% should be considered

(k) Manpower

For actual feeding operations four technicians should be considered. Each technician should be in charge of checking and feeding flies from two holding trolleys

(l) Additional equipment

Emergence cages and related recipients for puparia in incubation; one pupal sorting machine for central use and two chillers per nucleus colony for sexing of flies at emergence.

4.2. Biological parameters

Biological guidelines based on the performance of a 100 000 female colony are as follows:

1. The composition of the female population should approach the following picture: 15% flies younger than 18 days, with a mean daily mortality of 0.5%; 40% flies between the ages of 18 and 50 days, with a mean daily mortality of 1.2%; and 25% flies between the ages of 50 and 80 days, with a daily mortality of about 2%.

2. The male population should be about one third the size of the female population, with 75% of its numbers being between 5 and 30 days of age.

3. With a mean number of about 4250 puparia (from which less than 10% are below 24 mg) produced per 1000 mated females during 80 days, i.e. 0.053 puparia per initial female per day, about 5300 puparia per day or 150 000 per 30-day period will be produced.

4. If one assumes an overall emergence rate of at least 90%, 55% of which are females, about 2623 female and 2145 male flies will emerge daily. With an average daily mortality of 1155 females (or 34 650 per 30-day period), there will be a surplus of 812 females produced per day (24 360 per 30-day period). This number can be discarded (possibly already at the pupal stage with the aid of the pupal sorting machine) if the colony has to be maintained in the stationary phase. Another alternative solution would be to add those females to the colony and reduce the number of reproductive cycles for the entire colony.

5. If, at the most, one third of all emerging male flies are kept as studs for colony mating, the potential total output will be 48 240 males per 30-day period or 1608 males per day. This represents a factor of 0.016 males per day per initial female in the colony.

5. DISCUSSION

Some seven years ago, at a meeting on Control Programs for Trypanosomiasis and Their Vectors [22] held in Paris, an evaluation was made of the status on rearing of *Glossina*. One of the statements made was that 'There would seem little point in carrying out further research on rearing procedures using living hosts as the performance of colonised *Glossina* is already close to the limits imposed to the method of reproduction of the genus' (Citation, Section B, Actes du Colloque, p. 360).

Since then, experience in tsetse in vivo rearing obtained from laboratories in Europe has been successfully extended to Africa [7, 8], and leading laboratories in Europe (Bristol, Seibersdorf) have intensified their work on the use of artificial feeding methods. In vivo mass-rearing schemes in Tanga (Tanzania) and Bobo Dioulasso (Upper Volta) have shown that one of the main technical limitations is the maintenance of a large enough colony of healthy host animals. Workers involved in laboratory work using in vitro feeding techniques have proven the usefulness of the membrane system [3, 4] and results obtained so far open new perspectives.

Recognizing some of the limitations of the conventional in vivo rearing methods, e.g. cumbersome handling of small production units with limited number of flies, and being aware of some bottle-necks which still hamper large-scale implementation of the membrane feeding system, e.g. blood preservation methods, intrinsic risks of contamination, changes in the nutritional quality of the blood, we decided to pursue our research on those basic elements of in vivo rearing procedures that formerly constituted limits in the capabilities of the system.

The demonstration that larger cage types containing hundreds of flies can be used for production purpose and that adequate fertilization can be achieved with a minimal amount of stud males were essential features guiding our approach to improve rearing efficiency.

The introduction of cheap containers holding at least 200 mated females, and the use of handy mobile storage and feeding units, has enabled us to rationalize production and reduce the work load.

The results of our investigations on quality control aspects of the product produced, illustrate and confirm the practical usefulness of the pupal sorting machine for evaluating both quantitative and qualitative components of a mass-rearing operation. Its use enables a direct determination of the total number to be made, and an estimation of quality of puparia produced each day or during any

particular interval in a stock colony of a particular age composition. The quality is reflected by the frequency distribution of puparia over distinct weight classes that were previously defined during calibration tests. It is postulated that a stock colony in the stationary phase, maintained under standardized environmental and maintenance conditions, produces puparia with a stable frequency distribution pattern among the weight classes. Thus, any deviation from optimal maintenance conditions, e.g. too high temperature, too severe a challenge to the host animals, is likely to be immediately expressed (at least within the current reproductive cycle) by a shift in the frequency distribution of puparia towards the lower weight classes. It has been demonstrated that such changes will inevitably have an impact on the overall emergence rate, the sex ratio and, in particular, on overall fecundity when all freshly emerged flies are kept within the colony. Therefore, it is recommended that in order to keep the female stock colony in the stationary phase and to maintain a constant factor of fecundity, puparia collected in the lowest weight class(es) should be withdrawn or all newly emerged female flies from these classes should be destroyed.

The data presented on *G. p. palpalis* clearly demonstrate the reasonable potential of an in vivo rearing programme with the use of guinea pigs. It should be kept in mind that the margin between success and failure in colonizing a slow breeding insect like tsetse is very narrow. Manpower and equipment, including the blood donors, should be kept perfectly balanced. Under such conditions the tremendous capital investment may result in the desired pay off, i.e. economical production of adequate excess male flies to meet the requirements of a large-scale SIT campaign.

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LABORATORY MAINTENANCE OF *Glossina palpalis gambiensis* IN WEST AFRICA

Preliminary results of rearing on membranes

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Abstract

LABORATORY MAINTENANCE OF *Glossina palpalis gambiensis* IN WEST AFRICA: PRELIMINARY RESULTS OF REARING ON MEMBRANES.

A colony of *G. palpalis gambiensis* has been reared on membranes since October 1979. Cattle blood was used for feeding. The bood was collected from the local abattoir, defibrinated and irradiated. Before irradiation with a dose of 50 krad, 700 mg ATP and 1 g glucose per litre of blood were regularly added. The flies were fed daily. The colony performance was measured in terms of productivity, mean pupal weight and percentage of mean daily mortality. The flies derived from a colony fed on rabbits. A total of 28 760 females was transferred to the membrane facility. The results of the parental generation were poor. Their performance was characterized by a high daily mortality sometimes exceeding 6%, a low productivity rarely reaching 1 pupa per female in 30 days, and a distinct suboptimal pupal weight that was 3--4 mg lower than in the rabbit colony. The performance of the subsequent F_1- and F_2- generations followed a similar pattern. A slight improvement in the mortality was counterbalanced by a lower productivity, the mean pupal weight being as low as before. Neither colony reached a self-sustaining phase, and it was decided to return to a combined feeding regimen where the flies were fed five days weekly on membranes as before and twice weekly on rabbits. As a consequence all parameters measured showed distinct improvements. Since then the colony has reached an expanding phase. The irradiation dose of 50 krad was not only sufficient to prevent bacterial infections in the flies, but also interfered with the establishment of trypanosomes in the tsetse colony. No rabbit used for the mixed feeding regimen became infected, although it was estimated that on an average about 15% of the cattle arriving at the slaughter house were likely to be infected with trypanosomes.

INTRODUCTION

The successful application of the SIT in an area of 100 km^2 near Bobo-Dioulasso initiated the planning of a campaign in an area of about 2500 km^2. This area is situated south of Bobo-Dioulasso where three *Glossina* species occur:

TABLE I. *Glossina palpalis gambiensis* (ORIGINATING FROM INSECTARIES I AND II) FED ON DEFIBRINATED IRRADIATED CATTLE BLOOD, SUPPLEMENTED WITH ATP AND GLUCOSE

Periods of 30 days	Average female number/ day	Pupal production	Production/ female during 30 days	Pupal weights	Total mortality	
					Number	Percentage daily mortality
01.01.–30.01.80	2770	917	0.33	21.7	2316	2.79
31.01.–29.02.80	3047	587	0.19	21.9	3159	3.46
01.03.–30.03.80	5679	2027	0.38	23.1	11693	6.86
31.03.–29.04.80	5384	3281	0.61	23.0	8522	5.28
30.04.–29.05.80	6606	5050	0.77	24.2	12965	6.54
30.05.–28.06.80	4984[a]	5088	1.02	24.7	9081	6.07
29.06.–28.07.80	2241	2313	1.03	23.4	1501	2.23
29.07.–27.08.80	1066	759	0.71	22.0	967	3.02
28.08.–26.09.80	227	186	0.82	22.1	468	6.87
27.09.–26.10.80	32	27	0.84	18.3	47	4.90
			ϕ 0.67	ϕ 22.4		ϕ 4.80

[a] Last input of insectaries I and II (rabbits): 18.06.80.

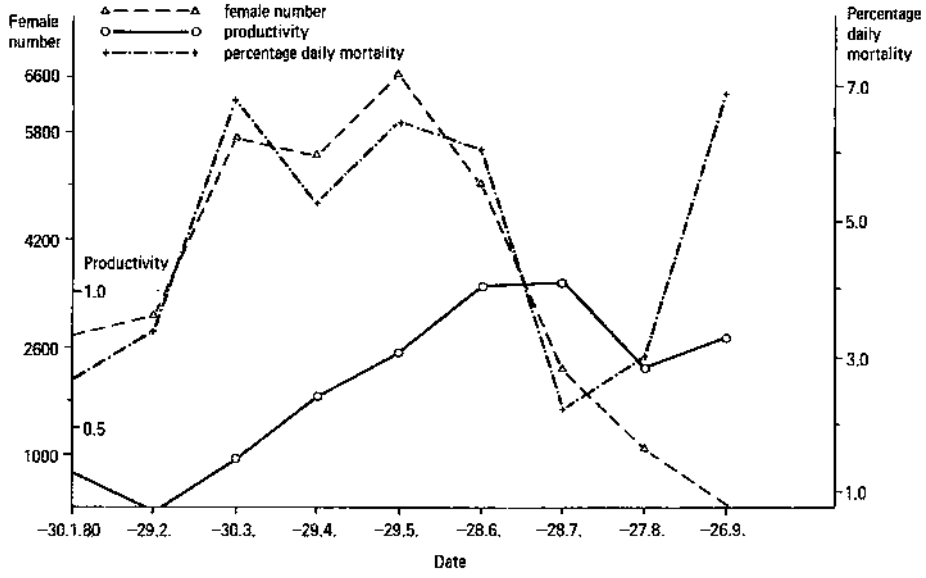

FIG. 1. Glossina palpalis gambiensis *fed on defibrinated irradiated cattle blood: Parental generation.*

G. palpalis gambiensis, G. tachinoides and *G. morsitans submorsitans.* The size of this area and the required simultaneous campaign against the three target species inspired a search for alternative methods of mass production, and experiments were undertaken to investigate the feasibility of a membrane-rearing system in Africa.

MATERIALS AND METHODS

Rearing on membranes was begun in October 1979 with *G. palpalis gambiensis.* Teneral flies were supplied from a colony fed on rabbits.[1] A total of 28 760 females and a similar number of males were transferred to the membrane system. The last input took place on 18 June 1980. Since then the colony has been closed. The fly cages consisted of PVC tubes, 6 cm high, 12.5 cm diameter, covered with terylene mesh netting. The membranes used were basically the same as described in Ref. [1]. The silicon base was constructed with a mould similar to the glass plates mentioned by Mews and co-workers [2]. The products

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TABLE II. *Glossina palpalis gambiensis* FED ON DEFIBRINATED IRRADIATED CATTLE BLOOD, SUPPLEMENTED WITH ATP AND GLUCOSE: F_1- AND F_2-GENERATIONS

Periods of 30 days	Average female number/ day	Pupal production	Production/ female during 30 days	Pupal weights	Total mortality	
					Number	Percentage daily mortality
31.03.–29.04.80	230	50	0.22	21.0	424	6.15
30.04.–29.05.80	546	144	0.26	21.2	805	4.92
30.05.–28.06.80	1170	626	0.54	23.0	1196	3.41
29.06.–28.07.80	1900	900	0.47	22.3	2033	3.57
29.07.–27.08.80	3854	2001	0.52	22.1	2673	2.31
28.08.–26.09.80	4196	3331	0.79	23.4	1784	1.42
27.09.–26.10.80	2852	1271	0.45	22.4	1822	2.13
27.10.–25.11.80	956	1174	1.23	22.0	793	2.77
26.11.–25.12.80	448	507	1.13	19.4	328	2.44
			ϕ 0.62	ϕ 21.9		ϕ 3.24

(RTV-M 540 and hardener T 46) were purchased from the Wacker Chemie GmbH, Munich. After use and cleaning, membranes and bases were placed together in an oven at 100°C for 16 h. The blood was routinely collected every week from various types of cattle in a local abattoir. Defibrination was achieved by stirring with a paddle. Thereafter the blood was stored at 4°C. The next morning the blood was bottled in 100-ml vials, ATP and glucose were added, and the irradiation took place in an irradiation chamber with a ^{137}Cs source. In contrast to the technique described in detail by Bauer and co-workers [3], 50 krad were used instead of 100, as the source emitted only about 1 krad/min, so that the blood had to remain 50 min in the irradiation chamber. If the blood had remained 100 minutes it would have been deleterious because of the ambient temperatures (often more than 30°C). In the beginning the blood was always bacteriologically checked. Repeated tests, however, revealed no or negligible bacterial growth, so that now bacteriological tests are only occasionally conducted. Tests with *Trypanosoma vivax* and *G. morsitans morsitans* were undertaken to find out the potential risk of infecting flies with trypanosomes from the blood of donor animals. Earlier investigations had shown that the overall infection rate of the cattle arriving at the abattoir amounted to 15%. *T. vivax* was used in the experiments because of its great potential for infecting flies. Blood was drawn from parasitized goats by puncture of the jugular vein with a hypodermic syringe. At this stage of parasitaemia the goats had $10^{7.5}$–$10^{7.8}$ *T. vivax* per ml of blood. The blood was defibrinated and prepared as mentioned earlier. Parts of the irradiated blood were microscopically investigated for five days. The remainder was offered to 50 teneral *G. morsitans morsitans*, males and females. For five consecutive days the flies had the opportunity to take up at least two infective blood meals. The experiment was terminated when the flies were 25 days old. Every fly was then dissected and controlled for trypanosomal infections.

The flies were kept at 85–95% relative humidity and 24°–25°C. However, because of failures in the power supply repeated climatic changes could not be avoided.

The colony performance was measured in terms of productivity, mean pupal weight and percentage of mean daily mortality. Flies older than 90 days were discarded.

RESULTS AND DISCUSSION

Table I and Fig.1 summarize the results from the parental generation. The overall performance of this colony was unsatisfactory when compared with the colony fed on rabbits. Whereas the productivity was always very low,

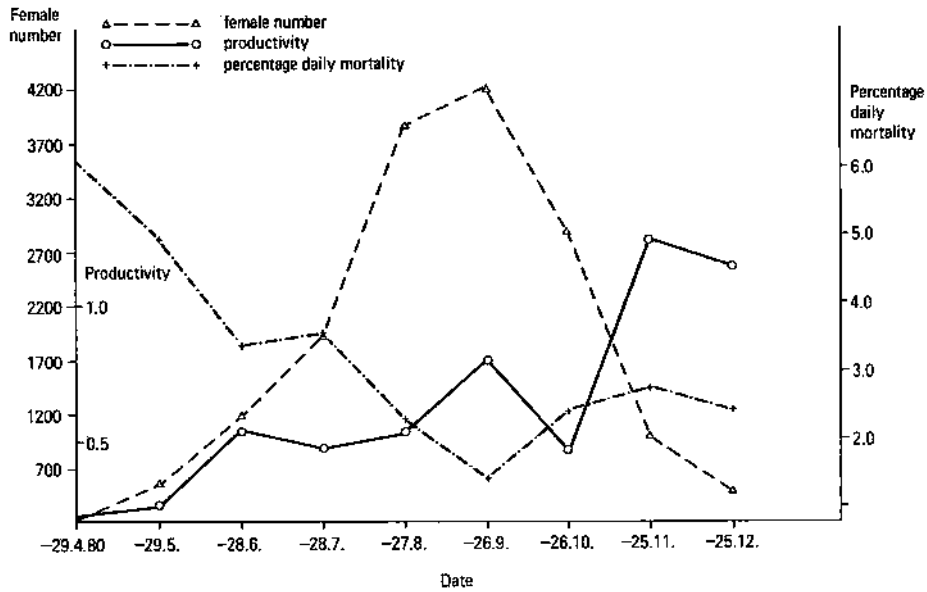

FIG. 2. Glossina palpalis gambiensis *fed on defibrinated irradiated cattle blood: F_1- and F_2-generations.*

reaching only a maximum of 1 pupa/female per 30 days, the percentage of daily mortality remained unacceptably high throughout the experimental period. Furthermore, the mean pupal weight never reached the level of the in vivo colonies. Most of the flies found dead were starved, showing that the blood uptake was insufficient. This was especially true for the first three age groups where the overall loss of flies amounted to 50%. The survival rate at the end of the 90 day period was only 10% at best. Summarizing the results, it is obvious that this colony could not reach a self-sustaining phase.

A total of 20 235 pupae being produced by the parental generation, it was thought that because of some adaptation the F-generations would show a better performance. The results of this colony consisting of F_1- and F_2-generations respectively are represented in Table II and Fig. 2. Again, it can be stated that the results of this colony were also suboptimal. Although the percentage of daily mortality was distinctly lower than in the parental generation it compared unfavourably with the in vivo colonies. In contrast to the parental generation the mortality during the first three age groups ranged between 20–30%. The survival rate at the end of the period amounted to 15%. However, the productivity was very low, and the mean pupal weight

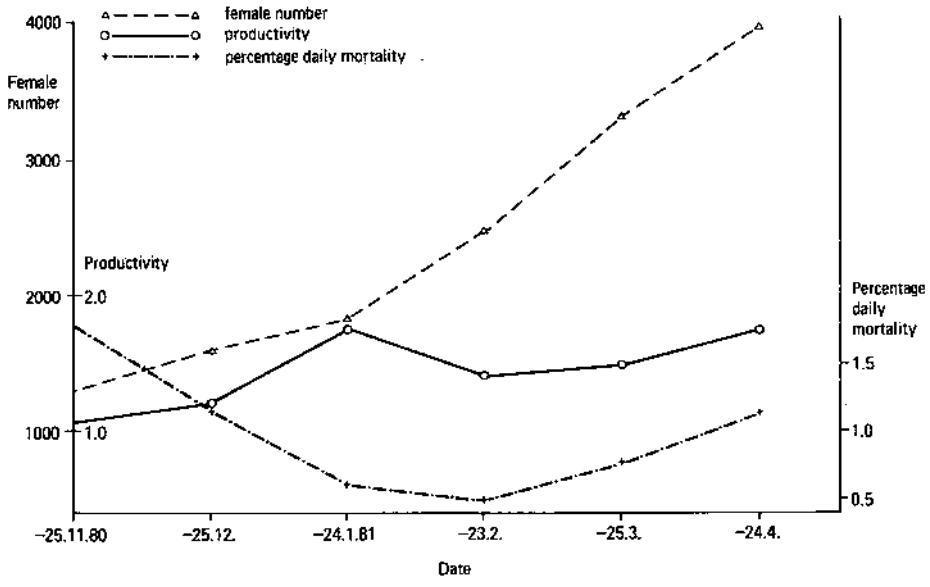

FIG. 3. Glossina palpalis gambiensis *fed on irradiated cattle blood 5 times weekly, on rabbits twice weekly: Descendants of F_1- and F_2-generations.*

remained suboptimal throughout the period of observation. Like the parental generation the first two filial generations never became self sustaining.

Because of these unsatisfactory results it was decided to go back to a combined in vitro/in vivo feeding regimen. The flies were fed on rabbits twice weekly (Wednesday and Saturday), and the remaining five days the flies were fed on membranes as before. The colony consisted of F_2- and subsequent generations. Table III and Fig.3 summarize the results. All three parameters measured show a distinct improvement compared with the preceding colonies.

This colony has reached a self-sustaining phase and the number of females is regularly increasing.

So far as the authors were aware, this is the first time that a *Glossina* species has been successfully maintained in Africa under the conditions described. Additionally, it has to be stated that *G. palpalis gambiensis* has never previously been successfully reared on membranes.

Taking into consideration the lapse of time required to maintain well-adapted species such as *G. m. morsitans* or *G. p. palpalis* on membranes in Europe a similar adaptation for *G.p. gambiensis* is not unlikely to occur.

In terms of the parameters measured it can be stated that under the mixed feeding regimen described the results resemble those obtained by the in vivo colonies.

TABLE III. *Glossina palpalis gambiensis* FED ON DEFIBRINATED IRRADIATED CATTLE BLOOD, SUPPLEMENTED WITH ATP AND GLUCOSE FOR 5 DAYS WEEKLY; 2 DAYS WEEKLY (WEDNESDAY AND SATURDAY) THE FLIES WERE FED ON RABBITS[a]

Periods of 30 days	Average female number/ day	Pupal production	Production/ female during 30 days	Pupal weights	Total mortality	
					Number	Percentage daily mortality
27.09.–26.10.80[b]	1069	433	0.41	22.9	440	1.37
27.10.–25.11.80	1290	1363	1.06	24.3	685	1.77
26.11.–25.12.80	1581	1903	1.20	24.2	541	1.14
26.12.–24.01.81	1820	3188	1.75	24.5	330	0.60
25.01.–23.02.81	2473	3452	1.40	24.6	365	0.49
24.02.–25.03.81	3307	4848	1.47	25.8	753	0.76
26.03.–24.04.81	3987	6921	1.74	26.9	1407	1.18
			ϕ 1.29	ϕ 24.7		ϕ 1.04

[a] This colony originated from the F_1- and F_2-generations.

[b] 2 days weekly on rabbits from 10 October 1980.

Up to now no bacterial infections have occurred in the flies thus indicating that the process of blood collection and the subsequent irradiation are sufficient for preservation of the blood. The irradiation did not visibly affect *T. vivax*. The trypanosomes were found motile even 5 days after treatment. However, no infections, either immature or mature, were found in *G. morsitans morsitans*. Thus it seemed likely that irradiation negatively affected the capability of the trypanosomes to adapt to the vector. This observation corresponded with the fact that none of the rabbits ever used for the mixed feeding regimen for *G. palpalis gambiensis* became infected.

Future work needs to emphasize the replacement of the rabbit supplement which might be simply a question of blood quality, and the reduction of fly handling procedures as an essential component of mass production.

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GENETIC APPROACHES TO THE STERILE INSECT TECHNIQUE

Session 5

LABORATORY EVALUATION OF THE LETHAL ALLELE *salmon* FOR GENETIC CONTROL OF THE TSETSE FLY, *Glossina morsitans morsitans*

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Abstract

LABORATORY EVALUATION OF THE LETHAL ALLELE *salmon* FOR GENETIC CONTROL OF THE TSETSE FLY, *Glossina morsitans morsitans*.

The lethal nature of the *salmon* allele in *Glossina morsitans morsitans* Westwood is confirmed at 23° and 25°C, and the lethality is demonstrated in two genetic backgrounds. Wild-type males lived longer than salmon males. There was no difference in longevity of wild type and heterozygous females but salmon females *(sal/sal)* did not live as long. Wild-type and heterozygous females produced approximately the same number of pupae but salmon females produced significantly fewer and at a lower rate than did wild-type females. Small cage experiments indicated that salmon and wild-type males were equally successful in mating with females when there was an excess of females. However, when there were twice as many males as females, the mating competitiveness of salmon males was about half that of wild-type males. In mixed populations there was assortative mating. In a population of heterozygous and salmon females the latter were found to be significantly less likely to be pregnant than the former. However, salmon males and wild-type males reared at 25°C do not differ significantly in their ability to impregnate females. A laboratory population under pressure of three salmon males to one 'field' male was 'eradicated' in eight generations and another population under 1:1 (salmon male : 'field' male) pressure had been suppressed at least 50% from the seventh generation onward. The *salmon* allele persists in a population and depresses the growth rate of the population for at least three generations after the release of salmon males into the population has been terminated.

1. INTRODUCTION

Studies on genetic control of tsetse flies have been concerned with dominant lethals produced by irradiation or chemosterilants, with production and use of chromosome translocations, and with the use of the natural genetic incompatibility between closely related species and subspecies [1–3]. Another approach would be to use deleterious alleles which would decrease the fitness of a population. One such allele in *Glossina morsitans morsitans* Westwood may be the lethal allele *salmon* (so designated because of the eye colour with which it is associated) [4]. The trait is controlled by an X-chromosome locus, is maternally influenced and is genetically rescuable. When females homozygous for this trait (*sal/sal*) were mated with hemizygous males (*sal*/Y), and

maintained at 23°C, about 80% of the offspring died in their puparia and most of the adults which did emerge died within a few days [4]. However the *salmon* allele is easily maintained in the laboratory in a colony of heterozygous females (+/*sal*) mated to salmon males.

As far as I am aware, *salmon* is the only lethal allele known in *G. m. morsitans*. The present study was undertaken to obtain laboratory data on longevity, reproduction and mating competitiveness of flies having the *salmon* allele, and to assess, under laboratory conditions, the potential of this allele as a genetic control agent.

2. MATERIALS AND METHODS

The origin and maintenance of our *G. m. morsitans* colony were described previously [5, 6]. Unless otherwise stated, all experiments were conducted in a room at approximately 23°C and variable but moderate relative humidity. In some experiments, the flies were maintained at 25°C, and relative humidity of 50% or greater, except for about 30 minutes on each of six days per week when the flies were at 23°C for feeding on rabbits. All experiments were conducted in cages which were 15 x 8 x 4.5 cm. All *salmon* flies used in these experiments were descendants of the original spontaneous mutation described previously [4].

3. EXPERIMENTAL PROCEDURES AND RESULTS

3.1. Confirmation of lethality

The lethal nature of the *salmon* allele was confirmed by maintaining populations of 40 to 46 mated females for 90 days at 23°C or 25°C. The results (Table I) show that the *salmon* allele was a conditional lethal at both temperatures and demonstrated that salmon females produced fewer pupae than did wild-type females. Although part of the reduced productivity was due to the shorter life of the salmon females (see below), part was due to reduced rate of pupal production. Pupae were harvested every two weeks and during 6 such periods at 23°C average productivity (pupae/female/day) was: 0.055±0.009 for *sal*/*sal* x *sal*/Y; 0.038±0.013 for *sal*/*sal* x +/Y; and 0.064±0.011 for +/+ x +/Y.

A colony was established consisting of females, homozygous for *ocra* and heterozygous for *salmon* (*oc sal*/*oc* +), mated to males having ocra bodies and salmon eyes (*oc sal*/Y)(see reference [4]). Only about one quarter of the autosomes in these flies came from the original *salmon* strain and at least some of the X-chromosome came from the *ocra* strain. To determine whether the *salmon* allele was still lethal, *oc sal*/*oc sal* females were mated to *oc sal*/Y males and pupae were collected. Only 4 males and 10 females emerged from 86 pupae; all had very pale eyes and died within a few days of eclosion.

3.2. Longevity of adults

In longevity experiments, the flies were observed every other day and dead flies were removed from the cages and their phenotypes

TABLE I. DEMONSTRATION OF LETHALITY OF THE *salmon* ALLELE IN *Glossina morsitans morsitans* AT 23° AND 25°C

Temperature and parental genotype	Pupae produced		Emergence number (% of expected)[a]	
	total	per female	Males	Females
23°C				
sal/sal X *sal*/Y	112	2.8	9(16%)	9(16%)
sal/sal X +/Y	92	2.3	11(24%)	36(78%)
+/+ X +/Y	180	4.1	76(84%)	77(86%)
25°C				
sal/sal X *sal*/Y	98	2.1	7(14%)	10(20%)
+/+ X +/Y	197	4.2	87(88%)	95(96%)

[a] Number expected was calculated assuming a 1:1 sex ratio and 100% emergence of both sexes.

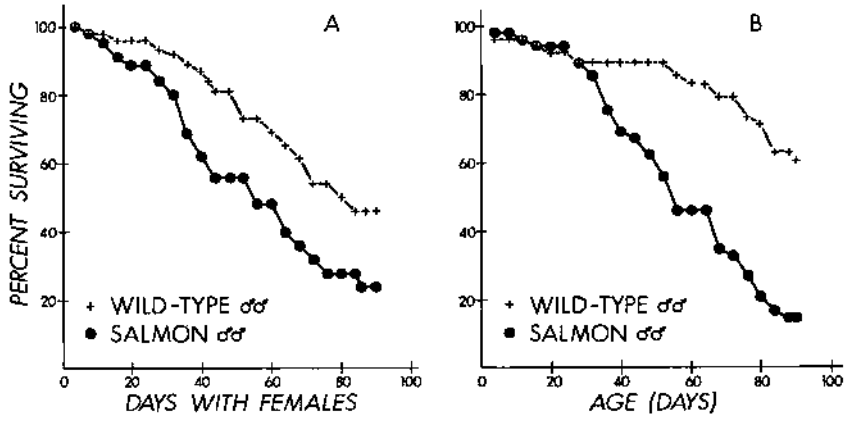

FIG.1. Longevity of wild-type and salmon male G.m. morsitans. *A, Males were approximately 8-days-old when placed with females (day 0 on graph). For the first 40 days, data are based on 46 wild-type and 45 salmon males; after this, data are based on an original population of 26 wild-type and 25 salmon males. B, Longevity of males in the absence of females. Data based on 48 of each type of male.*

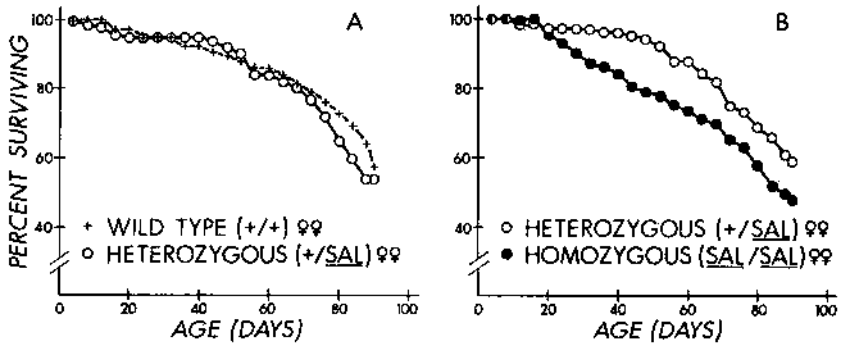

FIG. 2. Longevity of female G.m. morsitans. *A, Data are based upon 96 mated wild-type and 95 mated heterozygous females. B, Data based on 113 mated heterozygous and 111 mated salmon females.*

recorded. Longevity data, as cumulative percent mortality fitted to a smoothed curve, were analyzed using analysis of variance (ANOVA) [7–9].

The first observations on male longevity were made during the course of experiments in which 16 females were caged with two wild-type males and two salmon males. The results indicate that salmon males did not live as long as wild-type males (Fig. 1A; after 40 days $F=10.603$, $p<0.001$). In the second experiment, 6 cages were set up, each with 8 salmon males and 8 wild-type males. Again salmon males did not live as long as wild-type males (Fig. 1B; after 90 days $F=24.830$, $p<0.001$). The rate of decline of the population of salmon males was similar in the presence and absence of females but wild-type males died sooner in the presence of females.

Longevity of mated females was recorded in four experiments. Heterozygous females and wild-type females had similar life expectancies (Fig. 2A; after 90 days $F=0.004$, not significant), but salmon females did not live as long as heterozygous females (Fig. 2B; after 90 days $F=13.839$, $p<0.001$). Similar results were obtained comparing 69 heterozygous and 56 salmon females (after 90 days $F=7.743$, $p<0.01$). The longevity of homozygous salmon females was not influenced by whether they mated with salmon or wild-type males (data not presented here; after 90 days $F=0.335$, not significant).

3.3. Productivity of females

Pupal productivity by wild-type and salmon females at 23°C and 25°C is given above (Table I). A study of productivity by wild-type and heterozygous females mated with wild-type or salmon males indicated that pupal productivity was not markedly influenced by parental genotype (Table II). Although the results are consistent with an X-chromosome locus for *salmon*, fewer flies with salmon eyes were

TABLE II. PRODUCTIVITY OF 48 WILD TYPE (+/+) AND 48 HETEROZYGOUS (+/*sal*) *G. m. morsitans* FEMALES DURING 90 DAYS

	Genotypes of parents			
Females:	+/+	+/+	+/*sal*	+/*sal*
Males:	+/Y	*sal*/Y	+/Y	*sal*/Y
Pupae produced				
Total	169	179	235	165
Per female	3.52	3.73	5.00	3.44
Adult emergence				
Males (% of expected)[a]				
Wild-type	68(80%)	79(88%)	56(95%)	42(102%)
Salmon	0(N.A.)[b]	0(N.A.)	27(46%)	33(80%)
Females (% of expected)				
Wild-type[c]	77(91%)	85(95%)	85(72%)	40(97%)
Salmon	0(N.A.)	0(N.A.)	0(N.A.)	35(85%)

[a] Calculation of expected number was based on assumption of a 1:1 sex ratio and 100% emergence of both sexes.

[b] N.A. = not applicable.

[c] 'Wild-type' eyes include the genotypes +/+ and +/*sal*.

produced than expected. This might indicate that the *salmon* allele, in the homozygous or hemizygous state, is slightly deleterious before eclosion as well as after (see longevity data). (Tables III and IV provide additional evidence that *sal*/*sal* females produce fewer pupae than do heterozygous females.)

3.4. Mating competitiveness of males

The introduction of a deleterious allele into a natural population would probably be done by release of males carrying the allele. Therefore the mating competitiveness of the males carrying the allele ought to be assessed fairly early in the evaluation of any deleterious allele. Curtis [10] has reviewed the literature on mating competitiveness in tsetse flies and has commented upon the problems of estimating mating competitiveness. He pointed out that small cage experiments (particularly those involving several males and females) can over-estimate the competitiveness of the least competitive type of male. The limited amount of data published on mating competition between *ocra* and wild-type males indicates that they are equally competitive [11, 12]. One mating experiment has been carried out (10 replicates) using 12 males

TABLE III. MATING SUCCESS OF SALMON AND WILD-TYPE MALES WHEN PLACED WITH HETEROZYGOUS (+/*sal*) OR HOMOZYGOUS (*sal*/*sal*) FEMALE *G. m. morsitans*[a]

	Number of female offspring[b] produced by *sal*/*sal*		*+/sal*	
	Wild-type	Mutant[c]	Wild-type	Mutant[c]
Observed	57	69	152	64
Expected	63	63	162	54
χ^2	1.1429[d]		2.4692[d]	

[a] Each cage contained 16 females of one genotype, 2 wild-type males and 2 salmon males. There were 7 cages of each female genotype.

[b] All female offspring, those which emerged from puparia and those which died within puparia, were scored.

[c] For *sal*/*sal* the mutant offspring have very pale eyes whereas for +/*sal* the mutant offspring have salmon-coloured eyes (see Ref. [4]).

[d] 1 d.f. not significant at $p = 0.05$.

TABLE IV. MATING SUCCESS OF MALES IN PRESENCE OF BOTH HETEROZYGOUS AND SALMON FEMALES[a]

	Number of female offspring[b] produced by *sal*/*sal*		*+/sal*	
	Wild-type	Mutant[c]	Wild-type	Mutant
Observed	49	72	242	60
Expected	60.5	60.5	226.4	75.6

[a] Each of 10 cages contained 8 heterozygous (+/*sal*) females, 8 homozygous (*sal*/*sal*) females, 2 wild-type males and 2 salmon males. Females, at the black-lobed stage, were regrouped according to genotype before pupae were collected.

[b] All female offspring, those which emerged from puparia and those which died within puparia, were scored.

[c] For *sal*/*sal* the mutant offspring have very pale eyes whereas for +/*sal* the mutant offspring have salmon-coloured eyes (see Ref. [4]).

$\chi^2 = 8.6658$ (3 d.f., $p < 0.05$).

(6 wild-type, 6 salmon) per cage, to which 6 wild-type females were added. In individual replicates wild-type males mated with from 50 to 75% of the receptive females (mean=62.96±6.57%). Of 54 pairs which formed, 34 involved wild-type males and 20 involved salmon males (Chi^2=3.6296, 1 d.f., 0.1>p>0.05). Mating competitiveness has also been estimated by adding one female to a cage with one male of each type. Of 33 males which mated, 21 were wild-type and 11 were salmon (Chi^2=3.1250, 1 d.f., 0.1>p>0.05).

3.5. Mating success of males in presence of an excess of females

The behaviour of flies in small cages is no guarantee of how the flies will behave in nature. In the above experiments salmon males were estimated to be about half as competitive as wild-type males. If this is true in nature, then females entering a population consisting of 67% salmon males and 33% wild-type males would have an equal probability of mating with either of the two types of males. As long as the difference in relative mating competitiveness of the two types of males is not too great, one assumes that the composition of the population can be adjusted to obtain the desired probability of a female mating with any particular type of male. Starting with this assumption, and with the belief that small cage mating experiments involving several flies may produce variable and possibly unpredictable results, and with the knowledge that multiple mating occurs in *G. m. morsitans* [10–13], I conducted mating experiments in which there was an apparent excess of females. Basically female reproductive capacity is treated as a resource which may be utilized by salmon and wild-type males.

The relative ability of salmon males and wild-type males to utilize this resource was studied by placing 16 virgin females (3–7-days old) in a cage to which 2 salmon and 2 wild-type males (7–10-days old) were added. In seven cages heterozygous females were used, and in seven cages homozygous salmon females were used. Since *salmon* has an X-chromosome locus, only the female offspring indicate whether sperm from salmon or wild-type males were used. In the experiments in which salmon females were used, the phenotypes of unemerged females were determined and included in the calculations. The results (Table III) indicate that salmon and wild-type males were equally successful. These results are interesting in view of the shorter life expectancy of the salmon males (see above and Fig. 1), and indicate that all of the mating was done soon after the cages were set up.

The main conclusion that I wish to draw from these experiments is that, in doing a laboratory experiment on eradication of a population by release of salmon males, the probability of a female using sperm from salmon males will be directly related to the proportion of salmon males in the population, if the females are present in excess. Thus a population which has a salmon to wild-type ratio of 1:1, in the presence of an excess of females, would be equivalent to a salmon:wild-type ratio giving equal mating effectiveness when there is an excess of males. I suspect that mating competitiveness of salmon and wild-type males may vary with age of the flies and with such environmental conditions as light intensity, temperature and time since the last meal. Variations due to these effects might then be minimized by experiments in which there is an apparent excess of females.

This resource utilization was re-evaluated in an experiment which permitted assortative mating. Ten cages were set up, each containing 8 salmon females, 8 heterozygous females, 2 wild-type males and 2 salmon males. As females reached the black-lobed stage of their first pregnancy cycle they were regrouped in new cages, salmon females in one set of cages and heterozygous females in another. The F1 flies were sexed and the eye color recorded; pupae from which flies did not emerge were opened and the sex and phenotype of the dead fly was recorded. The data, from female offspring, indicate that there is assortative mating (Table IV). Neither the basis for the assortative mating nor its implications for use of the *salmon* allele have been evaluated yet. However, it must be pointed out that this assortative mating may have influenced the mating competition experiments since only wild-type females were used in those experiments.

3.6. Receptivity of females and insemination ability of males

In the assortative mating experiment described above, data were obtained on the receptivity of females. Of 80 heterozygous females, 69 were inseminated and reached the black-lobed stage, 7 never became pregnant and 4 were lost or accidentally injured during handling. The corresponding numbers for the 80 salmon females were 56, 18 and 6. Comparing the pregnant and non-pregnant females the contingency $Chi^2=6.2228$ (1 d.f., $0.01<p<0.025$).

A preliminary experiment was conducted to determine if salmon and wild-type males reared at 25°C differed in their ability to impregnate females. Of 82 females individually mated with wild-type males 74 were inseminated, as indicated by reaching the black-lobed stage, while 72 of 82 females were impregnated by salmon males. The former group of females produced 588 pupae (7.95 pupae/female) while those inseminated by salmon males produced 532 pupae (7.39 pupae/female).

3.7. Laboratory demonstration of eradication of *G. m. morsitans* by release of salmon males

Three populations were set up at 25°C. Each population began with 48 wild-type females evenly divided among 3 cages. Females were mated when less than one week old by placing in the cage one- to two-week-old males; the number of males was one half the number of females in the cage and the males were left with the females for 48 hours. Because I had no simple way of influencing fertility and fecundity in a predictable manner I simply increased or decreased the length of time adults were kept in order to increase or decrease the number of pupae collected, and hence the size of the next generation. The control population was managed so that it would undergo a population cycle approximately every 8 generations and the number of pupae collected when the population reached its peak was about 3.5x the number collected when the population was at its minimum. Collection of pupae for the experimental populations was terminated when the appropriate number of pupae had been collected to set up the next generation of the control population. No attempt was made to introduce a correction for the effects of density

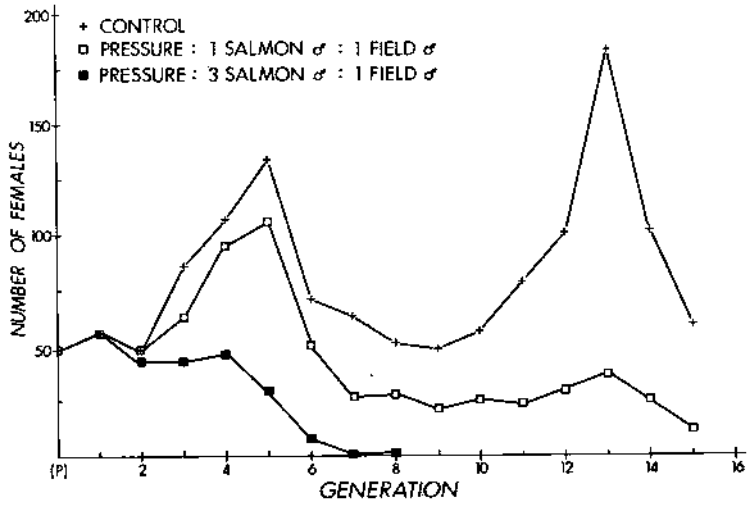

FIG.3. Laboratory demonstration of suppression of G.m. morsitans *population at 25°C by 'release' of salmon males.*

dependent factors on the growth of the experimental populations. In one experimental population the ratio of 'released salmon males' to 'field males' was 1:1, and in the second the ratio was 3:1. In setting up the first two generations, the salmon males came from the colony at 23°C but from the third generation onwards almost all males came from populations maintained at 25°C. Whenever I was short of males I used males from the colony at 23°C. Phenotypes of emerging flies in each generation were recorded and flies were mated as indicated above. All females which emerged during the week were used. In the experimental populations, 'field males' were selected in the ratio of phenotypes in that generation. From week to week there would inevitably be some discrepancy between the frequency of each phenotype in the populations and the frequency of the phenotypes of males used for mating. However, over all generations (from the third onwards), there was good agreement between the number of each phenotype used and the number which should have been used; for the 1:1 (salmon:field) population $Chi^2=0.2646$, 16 d.f., for the 3:1 (salmon:field) population $Chi^2=0.5204$, 10 d.f. The results (Fig. 3) show that after three generations the experimental population under pressure of 1 released salmon male to 1 'field' male was slightly smaller than the control population, while the experimental population which was under 3:1 (salmon male:'field' male) pressure was about one half the size of the control population. The latter experimental population was terminated in the eighth generation when only one pupa was produced. The population under 1:1 pressure was suppressed to about 50% of the control population by the seventh generation and remained at or below that level through the fifteenth generation.

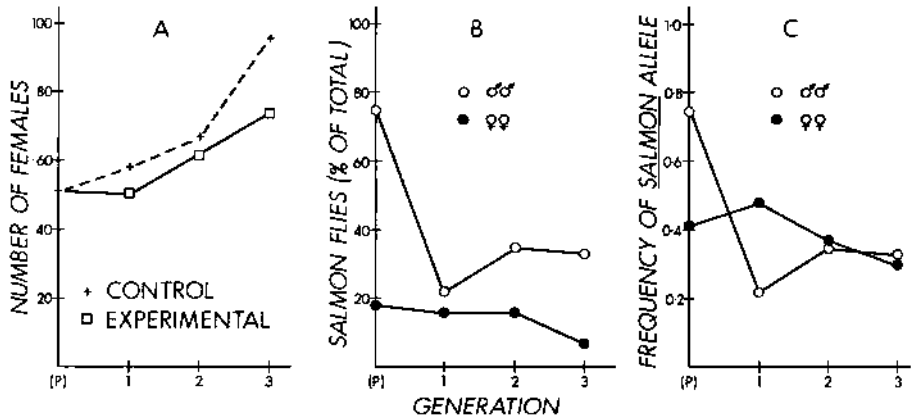

FIG. 4. Laboratory population of G.m. morsitans *after suspending 'release' of salmon males. A, Population growth. B, Observed frequency of salmon flies. C, Frequency of* salmon *allele observed in males and calculated for females.*

3.8. Persistence of the *salmon* allele in a population

One potential advantage of using a deleterious allele is that if, for any reason, the release program is suspended, the fitness of the population will be depressed until the population has eliminated the deleterious allele. A preliminary experiment on the persistence of the *sal/mon* allele in a population has been conducted. A control population of wild-type females (3 cages, each with 17 flies) was set up and managed so that the first three generations had 123, 160 and 227 pupae respectively. The parental generation of the experimental population consisted of 18 +/+ females, 24 +/*sal* females and 9 *sal/sal* females equally distributed among 3 cages. In the parental generation, the experimental colony had 6 salmon and 2 wild-type males placed in each cage for 48 hours. In subsequent generations, each week's emergence of females was mated with all the males which emerged during the previous week. Thus the control and the experimental populations were treated as closed colonies. In some weeks there was an excess of males during the 48-hour mating period, in other weeks there was an excess of females. The experiment was terminated after 3 generations, at which time the experimental population was 77% the size of the control population and was still growing at a slower rate (Fig. 4A). By the third generation 33% of the males were salmon, and the frequency of salmon females had declined from 18% to about 7% (Fig. 4B). However, among the females the frequency of the *sal/mon* allele was estimated to be 30% (Fig. 4C) during the third generation, indicating that the allele would have persisted for several more generations, had the experiment been continued.

4. DISCUSSION

The *salmon* allele adversely affects several aspects of tsetse biology: very few adults emerge from puparia produced by mating salmon females with salmon males, and most of these adults die within a few days; the longevity of salmon females and of salmon males is less than that of wild-type flies; salmon females produce offspring at a lower rate than wild-type and heterozygous females; and salmon females are less likely to become pregnant than are heterozygous females. All of these changes in the life of the flies would depress the fitness of a population having the *salmon* allele.

There is assortative mating, i.e. in a mixed population salmon flies tend to mate with each other and wild-type flies tend to mate with each other. The implications of this have not yet been assessed.

Not all aspects of the biology are adversely affected by the *salmon* allele: heterozygous females live as long as, and produce as many offspring as, wild-type females, and the *salmon* allele is easy to maintain in the laboratory.

There are, however, several reservations about using the allele *salmon* as a biological control agent. In small cage experiments, the mating competitiveness of salmon males is only about half that of wild-type males and this may create problems in introducing the allele *salmon* into a natural population. In practice we know nothing about how the salmon flies will behave when released in nature. However, the salmon flies which escape in the laboratory behave superficially like wild-type flies, i.e. they fly to windows and fluorescent lights (including a fluorescent ultraviolet light) and they return to bite us from time to time. One obstacle to further consideration of this allele is simply a question of how efficient this system would be when compared to the sterile male release program. For example, with our present techniques only half the males produced by a colony have salmon-coloured eyes. We are working on ways to overcome some of the drawbacks and studies are continuing on various aspects of the biology, genetics, physiology and biochemistry of flies having the *salmon* allele.

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POSSIBLE GENETIC SEXING MECHANISMS FOR *Ceratitis capitata* Wied.

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Abstract

POSSIBLE GENETIC SEXING MECHANISMS FOR *Ceratitis capitata* Wied.

Genetic methods for separating the sexes have been developed for a variety of dipteran insects. Special consideration is given here to techniques based on making males resistant to a toxicant to which females remain susceptible, a situation which is accomplished by linking an appropriate resistance gene to the Y-chromosome with a translocation. The choice of toxicant depends above all on the isolation of a resistance gene. Progress is reported on the early stages of developing techniques for *Ceratitis capitata* based on ethanol tolerance and dieldrin resistance.

1. INTRODUCTION

The advantage of removing females from cultures of insects intended for release is readily appreciated. Insects are very expensive to rear, and the removal of females would lead to a substantial saving in space, in the cost of rearing and of irradiation, packing and distribution, so increasing the capacity of the breeding facility. The potential saving in costs could be very considerable; estimates for several species given by LaChance [1] range up to US$4—7 million/year (for screwworms). For *Ceratitis capitata* the potential saving is now estimated to be at least US 2 million/year for the Mexican MOSCAMED programme alone [2]. The need to remove females of *C. capitata* is particularly pressing because large numbers of them, even when sterilized, can cause extensive damage. The punctures they make with their ovipositors produce blemishes on the fruit reducing its market value.

2. CHOICE OF TECHNIQUE

Because of the advantage of removing females, considerable interest is now centred on developing suitable techniques for accomplishing this. The methods theoretically available can be classified into two broad categories: prezygotic and

TABLE I. GENETIC SEXING MECHANISMS
Some examples

Species	Mechanism	Efficiency (%)	Reference
Musca domestica	Crosses between strains with different sexual formulae	100	[3]
	Male-generating gene *(Ag)*	100	[4]
	Heat-sensitive, m-linked recessive lethal	100	[5]
Aedes aegypti	Meiotic drive at M^D locus	93–94	[6]
	M^D + T_1 translocation	97–99	[7, 8]
Culex tritaeniorhynchus	Heat-sensitive lethals	99.3 100	[9] [10]
Ceratitis capitata	Y-translocated pupal colour gene	84–98	[11]
Drosophila melanogaster	Y-translocated ethanol resistance gene	100	[12]
Anopheles gambiae	Y-translocated dieldrin resistance gene	98–99	[13]
Anopheles albimanus	Y-translocated propoxur resistance gene	98–99	[14]

postzygotic. Prezygotic techniques include meiotic drive genes and autosomal sex determining factors (examples in Table I). These act at meiosis or in the gametes. Their early action makes them highly convenient to use, and they can be very efficient. However, they have been developed only in species like *Musca domestica* and *Aedes aegypti* [3, 4, 6–8] which are relatively well studied from a genetic point of view. Such genes might turn up in the course of genetic study on *C. capitata*. However it would almost certainly prove unprofitable to try to produce them 'to order' as Foster and Whitten [15] found when they tried unsuccessfully to isolate meiotic drive genes in *Lucilia cuprina*.

Postzygotic techniques include heat-sensitive recessive lethals, Y-translocated genes giving resistance to a toxicant and induced sexual dimorphism (examples in Table I). Some of the more efficient methods can be made to act early in development (on eggs or first instar larvae) which is clearly an advantage.

Three genetic sexing strains have been made using heat-sensitive lethals, one in *M. domestica* [5], the other two in the mosquito *Culex tritaeniorhynchus* [9,10]. In each case it was necessary to employ mutant markers and inversions to screen for the appropriate lethals associated with the female-determining chromosomal element. This approach to genetic sexing does not seem a practical proposition for *C. capitata* at the present time because of insufficient basic information on markers and cytogenetics. However, it may become possible to devise such a strain in the future.

Genetic studies on *C. capitata* have been pioneered by Rössler [11] who is reporting in this Symposium[1] on his technique based on a morphological colour difference in the pupae (Table I). He has succeeded in translocating an autosomal colour gene into the Y-chromosome so that the male pupae are brown and the female pupae black. This experiment has been important in demonstrating the feasibility of Y-autosomal translocations in this species. It also revealed the existence of recombination between the Y-chromosome and the colour locus (Rössler, personal communication) so that a proportion of black male pupae and brown female pupae are produced in each generation. Rössler's experience thus points to the need to incorporate into any future sex-separating systems based on a Y-translocation a means of inhibiting recombination such as an appropriately sited inversion.

Y-translocated insecticide resistance (Table I) is another postzygotic technique. In the mosquito *Anopheles gambiae*, Curtis and co-workers [13] demonstrated the feasibility of translocating a gene for dieldrin resistance on to the Y-chromosome, the males thereby becoming resistant and the females remaining susceptible. In *Anopheles* mosquitoes resistant to dieldrin is normally controlled by a single semi-dominant autosomal gene. Resistant males were irradiated and crossed, and backcrossed to susceptible homozygous females. Curtis and co-workers selected a family in which the gene for dieldrin resistance had been translocated on to the Y-chromosome so that males were heterozygous for resistance and females were susceptible homozygotes (Fig.1). Thus the females could be selectively killed by dieldrin treatment at the first larval instar, leaving the males unharmed. This genetic sexing strain was self perpetuating.

The same general approach was followed by Keiser and co-workers [14] with a different *Anopheles* species and a different insecticide. However, they introduced a further refinement, a sex-linked inversion, which was very successful in reducing recombination to a minimum (Table I). This kind of approach seems the ideal one to adopt for *C. capitata*, to make males resistant to an easily used toxicant, to which females remain susceptible, by translocating a gene-conferring resistance to the toxicant on to the Y-chromosome, and then adding an inversion to reduce recombination between resistance and sex to a minimum.

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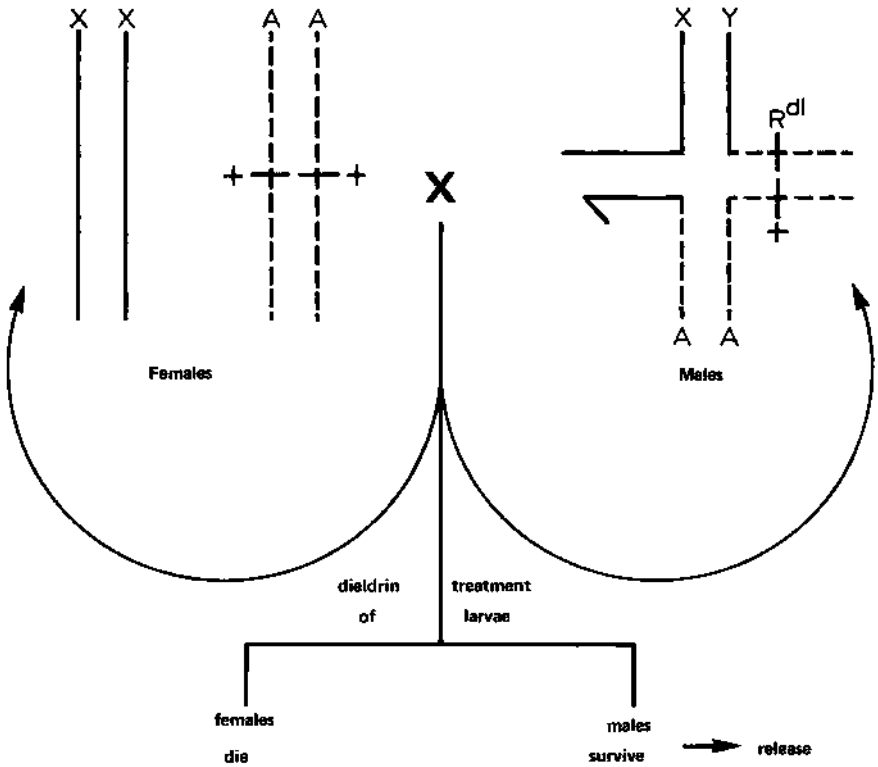

FIG. 1. Genetic sexing technique based on a Y-translocated dieldrin-resistance gene (R^{dl}): *A = autosome.*

3. CHOICE OF TOXICANT

There is a strongly held opinion against the use of insecticides in genetic sexing. This is on grounds of (a) possible contamination, (b) possible release of a few semi-fertile resistant males. Contamination is undoubtedly a potential danger, for which it would be necessary to develop a strict code of practice. However, experience with *Anopheles albimanus* and propoxur [14] suggests that the danger can be contained. The release of resistant males is no problem unless the insecticide used (or a related compound) is an actual or protential control agent. Even then, the chance of resistance being introduced by such a heavily irradiated parent seems slight.

Apart from the above considerations, any toxicant used in the breeding facility must have certain essential properties. It must be readily available, easily handled and preferably inexpensive. But above all the toxicant must be one to

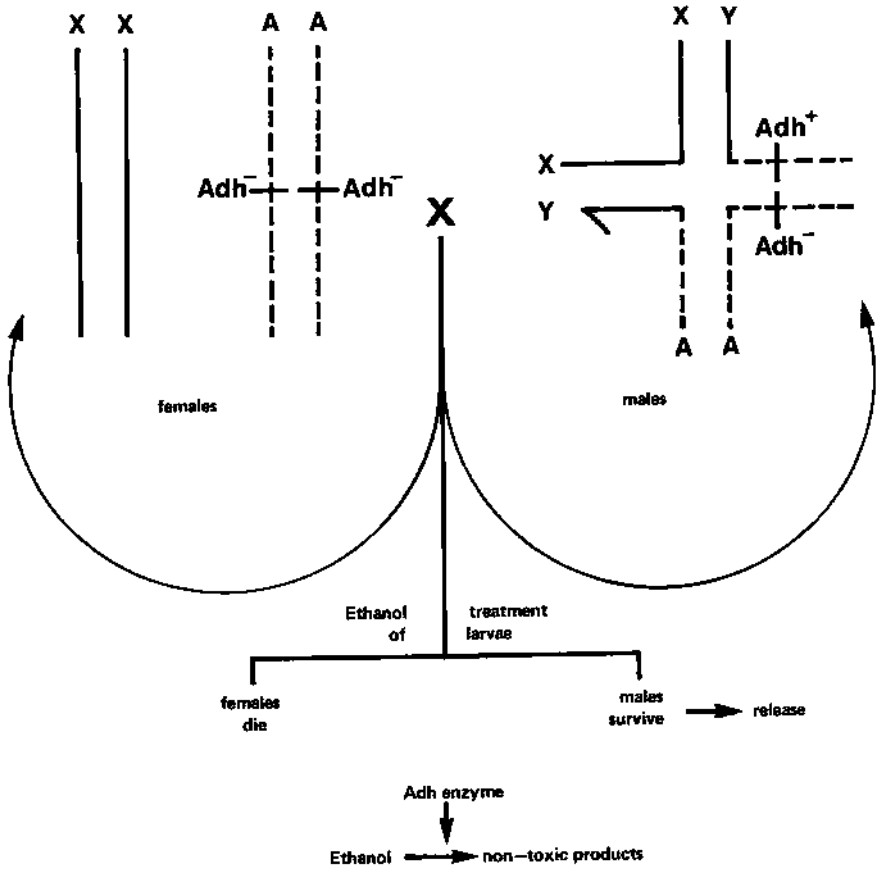

FIG.2. Genetic sexing technique based on a Y-translocated gene (Adh^+) *giving resistance to ethanol. A = autosome.*

which insects are known to vary genetically in tolerance and for which the basis of variation is a single major gene. All these considerations have led us to opt for the cyclodiene insecticide dieldrin as a possible candidate for *C. capitata*. To investigate the potential for dieldrin resistance we have put third instar larvae of a Costa Rican strain under strong selection. The response was immediate and the form of the LC-p dose response curves indicated a genetic basis for high resistance. The details will be considered later.

As a second possible toxicant we favour the use of ethanol. Experience with ethanol for separating the sexes of *Drosophila melanogaster* [12] is certainly encouraging (Table I). The principle is shown in Fig.2. Tests on *C. capitata* with ethanol reveal a high natural resistance by larvae of all strains. The first step

therefore is to produce a susceptible strain. There are two possible ways of achieving this, each of which is based on the assumption that resistance is due to a detoxifying enzyme alcohol dehydrogenase (ADH), the absence of which leads to susceptibility (as is the case in *D. melanogaster).*

The first approach is to screen for *Adh*$^-$ ('null') mutations after EMS treatment using electrophoresis. This method is currently being followed by A.S. Robinson and C. van Heemert in Wageningen (personal communication).

The second approach, one which we have followed, is to select with the secondary alcohol pentenol. Studies with *D. melanogaster* [16] have shown that resistance to ethanol is associated with susceptibility to pentenol, and vice versa. This is because the enzyme ADH which breaks down ethanol to relatively non-toxic products, breaks down pentenol to a highly toxic ketone. Thus if selection leads to an increase in resistance to pentenol, and *if this is due to a loss of capacity to manufacture ADH,* the result should be an increase in sensitivity to ethanol. Progress so far will be considered below.

4. EXPERIMENTAL PROCEDURE

The procedure for developing a genetic sexing technique can be divided into three phases:

(a) The production of strains resistant and susceptible to the toxicant,
(b) Genetic analysis to determine mode of inheritance of resistance,
(c) The synthesis of a genetic sexing strain by inducing an appropriate translocation and inversion.

4.1. Production of resistant and susceptible strains

This requires techniques of bioassay and possibly electrophoresis. It is important to note that the technique ideal for developing resistant and susceptible strains may not be the same as the exposure method finally employed for killing females in mass culture. Thus we have found it convenient to use third instar larvae for bioassay, both with dieldrin and with pentenol/ethanol, although it is hoped finally to develop a technique for killing the females as first instar larvae or eggs.

After experimenting with various methods of exposure we have chosen to use a total immersion technique. The third instar larvae are immersed in batches of 50: in 3.125–1600 ppm dieldrin in de-ionized water dispersions for 1 h; in 0.7–5% pentenol for 3 h; and in 86% ethanol for 6–24 h.

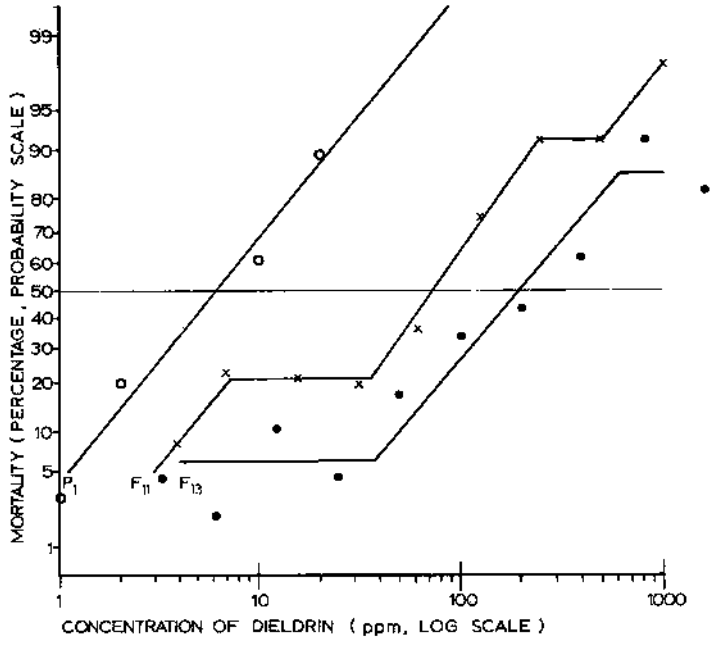

FIG.3. Response of the Costa Rican strain of C. capitata *to selection with dieldrin.*

4.1.1. Dieldrin selection

A strain of Costa Rican origin selected with dieldrin showed an increase in LC-50 from 6 ppm in P_1 to 50–70 ppm in F_{11} and to 200–211 ppm in F_{13} (resistance ratio 33–35). The range in LC-50, and resistance ratio, reflects the two ways in which the data may be plotted, either as a straight line or a stepped line, as in Fig.3. The latter is undoubtedly a more accurate representation of the data, and probably indicates segregation of the three genotypes RR, R+, ++. On this interpretation the frequency of RR has risen from 8% in F_{11} to 16% in F_{13}.

We have also investigated a strain obtained from Southampton University which shows a high natural resistance to dieldrin, the R gene being present already at high frequency (LC-50 of F_2 = 315–480, resistance ratio = 52–79). This line is being further selected, in parallel with the Costa Rican strain.

The baseline used for calculating resistance ratios is the unselected Costa Rican strain (LC-50 = 6 ppm). However, this strain is certainly not homozygous susceptible (we should not have been able to select for resistance if it had been), and it shows a rather flat regression line (Fig.3). A programme of single family sib selection of this line is now in progress to isolate a homozygous ++ strain, to

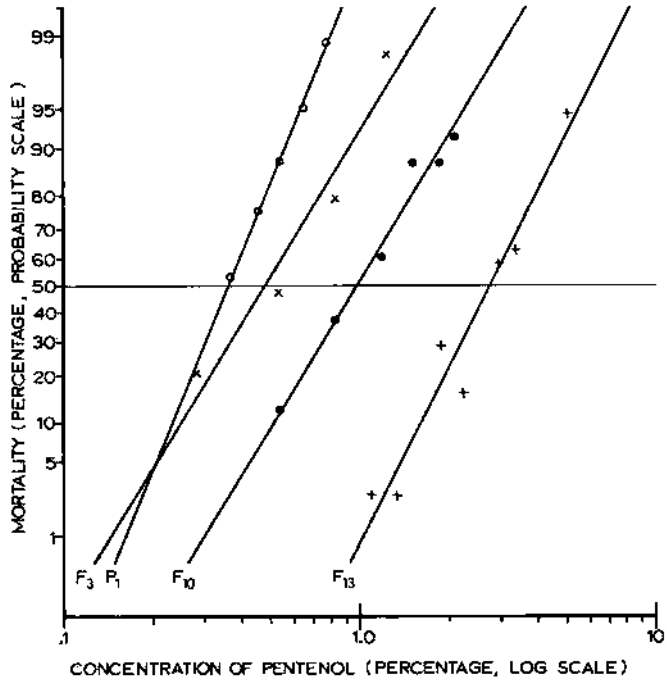

FIG.4. Response of the Southampton strain of C. capitata *to selection with pentenol.*

provide a true baseline for our tests. In sib selection [17], half the progeny from a series of single pair matings are tested for resistance, and the untested halves of those families which prove to be most susceptible are used for further breeding. Progenies of single pairs taken from the Costa Rican strain have been tested with 25 ppm dieldrin. Considerable variation was observed, and families with low survival have been individually reared with the intention of producing a homozygous susceptible strain.

A similar approach is being applied to producing a homozygous resistant strain because of slow progress in reaching homozygosity with mass selection. The advantage of sib selection is that the new generation is obtained from unexposed insects, a great benefit in the case of dieldrin, the toxic effect of which is partly delayed.

4.1.2. Pentenol selection

The effect of pentenol selection on four strains (from Costa Rica, Italy, France and Southampton University) has been to demonstrate that there is indeed

natural variation in response to this chemical. In Italy, the resistance ratio has reached 7 by F_{15}, in France 7 by F_{13}, in Costa Rica 5 by F_{15} and in Southampton 8 by F_{13} (Fig.4). All these estimates are based on ratios of LC-50s.

We compared the Southampton pentenol-selected line with the unselected Southampton strain for tolerance to ethanol. This line, which had increased 3 times in tolerance to pentenol in 7 generations, showed a decrease in tolerance to ethanol of 2–3 times (LT-50 to 86% ethanol from 9 h to 3.5 h). It seemed reasonable to conclude therefore that selection with pentenol had brought about a decrease in ADH activity as predicted. However, more recent tests with ethanol, both on eggs and third instar larvae, have given less clear-cut results. At the time of writing, we are not in a position to draw a final conclusion about the effect of pentenol selection on ethanol tolerance. Selection with pentenol is being continued.

4.2. Genetic analysis of resistance

The genetic analysis of resistance is not expected to pose any great problems. However, it is possible to obtain resistance and find, after carrying out the appropriate crosses, that it is genetically unsuitable for the construction of a sexing strain. What is needed is a dominant or semi-dominant autosomal or incompletely sex-linked gene. It is vital that a single diagnostic dose of insecticide can be used to separate the heterozygotes from the susceptible homozygotes, all the latter being killed and all (or most) of the former surviving. The chance of getting a holandrically inherited mutant is minimal, but this would of course be ideal (removing the need to induce a translocation). The chance is increased in a species with multiple sex-determining mechanisms like the housefly. Almost nothing is known about *C. capitata* in this respect.

4.3. Synthesis of a genetic sexing strain

Having produced strains homozygous resistant and susceptible for a suitable resistance gene (either to dieldrin or ethanol), the aim is to translocate the resistance gene on to the Y-chromosome. This would be done by (a) irradiating resistant males to induce translocations, (b) crossing the irradiated males to virgin susceptible females, (c) backcrossing F_1 males to virgin susceptible females in single pairs, (d) isolating families with suitably Y-translocated resistance genes.

The backcross families would need to be kept separate because each family would be descended from a single irradiated spermatozoon. The percentage hatch of the backcross eggs would provide a guide to whether a translocation had been induced. The fertility of translocation heterozygotes would be expected to range between 40–60% compared with 96–97% in the unirradiated controls.

Isolation of suitable Y-translocations would be achieved by treatment of newly hatched larvae with the appropriate diagnostic dose of the toxicant [13], or by incorporating it in the larval medium [12]. If eggs should prove to be more tolerant to the toxicant than larvae, it would be important to ensure that all eggs have hatched before treatment [13]. Families with a suitable translocation would contain all or mainly males.

The presence of a minority of resistant females in such families would be an indication of recombination between the resistance gene and the male determining element.

It would be necessary to minimize this because it would lead to an increasing number of resistant females in future generations. The aim would therefore be to isolate a suitable inversion using a breeding scheme identical to that given above except that the irradiated males would already carry Y-translocated resistance

Either X-rays or gamma rays would be suitable for inducing the translocations and inversions, the dosage chosen being one sufficiently high to cause the kind of change required but not so high as to produce total sterility. This is the two-hit dose range which is usually equivalent to 85–95% sterility for the young males of most Diptera. Rössler [11] was successful in inducing translocations with 1.2 krad (^{60}Co source) at the mid-pupal stage. Pupae are convenient to use because they require no anaesthetic. Rössler (personal communication) has found 1 krad to pupae causes 43–75% male sterility, and 2 krad causes 92–97% sterility compared with 3–4% sterility in unirradiated controls. Newly emerged adults would require a higher dose; 5 krad has been successfully used by Robinson and Van Heemert (personal communication) to induce translocations.

5. CONCLUSION

The production of strains resistant and susceptible to a suitable toxicant is the first hurdle to overcome, and probably the major one, in developing a genetic sexing procedure for *C. capitata.*

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GENETIC SEXING OF INSECTS BY PHENOTYPIC CHARACTERISTICS, WITH SPECIAL REFERENCE TO THE MEDITERRANEAN FRUIT FLY

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Abstract

GENETIC SEXING OF INSECTS BY PHENOTYPIC CHARACTERISTICS, WITH SPECIAL REFERENCE TO THE MEDITERRANEAN FRUIT FLY.

Genetic sexing is the induction and use of inherited, sex-limited characters to separate automatically males from females in mass-reared insects. Morphological as well as biochemical traits are used. The use of morphological traits in holometabolic insects is most advanced in the pupal stage, which is also amenable to various sexing methods. In insects with heterogamic sex-determination systems (such as Diptera and Lepidoptera), Y-autosome translocations are used to link the 'sexing' character to the sex-determination system. The method is discussed in detail, with special reference to the Mediterranean fruit fly. The latter employs an XY, XX sex-determination system. The effect of constructing a 'genetic sexing' line on its sexual competitiveness is discussed. The integrity of Y-autosome translocation lines depends on the natural absence or artificial suppression of genetic recombination on the 'translocated' chromosome. Preliminary studies revealed the existence of genetic recombination in the medfly males. The consequences of that on the construction of 'genetic sexing' lines in that species is discussed.

THE SCOPE OF GENETIC SEXING

The term 'genetic sexing' is used in the context of the Sterile Insect Release Method (SIRM) and denotes the use of inherited characters to separate males from females during the process of mass production. The separation has to be efficient and automated and is followed by the elimination of one sex or the retention of both sexes separately for further use.

The incorporation of an efficient and automated sexing method into the mass-rearing process has many advantages in most SIRM projects and is indispensable in some. Males are considered in most, if not all, SIRM projects as the desired sex for release. Females 'range from being a definite liability to a

useful asset' [1]. Sterile females of various mosquito species remain vectors of diseases, and females of biting flies will cause weight loss and be a nuisance to cattle. Sterile fruit fly females continue to oviposit their sterile eggs and produce ovipunctures which cull the fruit eventually [2–4]. Boll weevils and screwworm females require a higher sterilization dose than the males. Thus males have to be overdosed to ensure the complete sterilization of the accompanying females [2]. Females could also interfere with the normal dispersal of the sterilized males in the field [5].

Genetic sexing and the eventual elimination of the females during the rearing process will also result in cost-saving as the handling of material is halved. Additional benefits accrue from the fact that when only one sex is released, the need for marking the released insects is eliminated. Last but not least, Kojima [6] and Whitten and Taylor [7] discussed the dynamics of SIRM, and showed that separate releases were more effective than the release of mixed populations.

There is no doubt that genetic sexing is an important and indispensable part of any SIRM project. The goal of genetic sexing is to eliminate one sex or separate and retain both sexes. Sex-limited conditional lethals are used for the elimination of one sex. (This is the topic of another paper in this symposium.[1]) Usually, biochemical or physiological mutants, such as sensitivity to chemicals or environmental conditions, are utilized for sex elimination. But, in some cases, morphological mutants may also serve as 'conditional lethals'. An example are the 'topaz' and 'white' eye mutations in the sheep blowfly, *Lucilia cuprina* (Wied.), which render their bearers non-functional in the field, but do not affect their performance in the laboratory [8]. Potential morphological characters that could be used in sex-eliminating systems are mutants affecting wing shape or size, and determine the ability of the released flies or insects to survive and move in the field.

Morphological characters will more likely be used not for sex elimination but for sex separation and the retention of both sexes for further use. They can be divided into three general categories: colour, size and weight. Some of the characters are normal secondary sex characters and others have to be sex linked through genetic manipulations.

Sex-limited, morphological characters, which can be used efficiently for the automated sexing of large numbers of individuals, are present or can be induced in all the pre-imaginal stages as well as in the adults of the target population (Table I). Silk-worm breeders developed special strains, carrying chromosomal translocations, where colour markings were linked to the sex chromosome of the heterogametic female [9]. These strains produced dimorphic eggs, larvae or cocoons which enabled the breeders to separate sexes by colour [10].

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TABLE I. GENETIC SEXING BY MORPHOLOGICAL CHARACTERS OF USEFUL INSECTS AND PESTS

Stage	Character	Insect species	Source
Egg	Colour	*Bombyx mori* L.	[10]
Larvae	Colour	*B. mori* L.	[10]
	Rate of development	*Culex pipiens fatigans* Say	[11]
Pupae	Size	*C. p. fatigans* Say	[13]
		Aedes aegyptii (L.)	[14]
		Anopheles albimanus Wied.	[15]
		Rhagoletis cerasi L.	Russ (personal communication)
	Colour	*Lucilia cuprina* (Wied.)	[4]
		Ceratitis capitata Wied.	[17]
		B. mori L.	[10]
Adults	Weight	*Musca domestica* L.	[16]

Sexing of silk-worm for directed crosses prior to adult emergence is essential, as adults tend to mate upon emergence. The developmental period of larval stages of mosquitoes was used during the WHO/ICMR SIRM project in India to sex *Culex pipiens fatigans* Say [11]. Male larvae of that species have a shorter development period than females, and cropping pupae during the first two days of pupation, combined with sexing by size, produced pupae which were 95–98% males [12]. The pupal stage is the natural candidate for genetic sexing by morphological characters. Pupal size, weight or colour could be, and were, used repeatedly for sexing of insects. Pupal size, which in some insects is a secondary sex character, has been used to separate sexes of mosquitoes such as *C.p. fatigans* Say [13], *Aedes aegyptii* (L.) [14] and *Anopheles albimanus* Wied. [15]. Pupal size has been used also for the sexing of the cherry fruit fly, *Rhagoletis cerasi L.* (Russ, personal communication). Pupal colours are usually the phenotypic expression of single gene mutations, which may be autosomal or located on the sex chromosome. In most cases they have to be linked to the sex-determining factors by some genetic manipulation. The genetic manipulation involves usually the construction of a translocation between the chromosomal segment carrying the mutation and the hetero-sex chromosome. The weight of the adult houseflies was used to separate males from females by passing a regulated

air current through a mass of anasthetized flies. Males, which were somewhat lighter than females, were carried by the air current above an artificial barrier to be collected for further use [16].

CONSTRUCTING GENETIC SEXING SYSTEMS WITH SINGLE GENE MUTATIONS

In developing a genetic sexing system we have to start with a suitable character that could be used in an automated system. The character could be a conditional lethal or a morphological character with clear-cut distinction between the phenotypic expression of the two alleles. The next essential step would be to link the character to the sex-determining system in such a way that the line will produce continuously and with reliability two morphs, each for each sex. An example would be the production of males with brown pupae and females with black pupae, each and every generation [4, 17].

When the sex-determining system involves a homogametic and a heterogametic sex, and sex is determined by the presence of a hetero-chromosome and not by the balance between autosomes and sex chromosomes, the technique for linking the 'sexing character' to sex becomes rather simple, and is based on translocating the piece of chromosome carrying the 'sexing character' to the hetero-chromosome by the following scheme: (1) Irradiate wild-type males; (2) Cross the irradiated males with mutant females; (3) Separate the F_1 males; (4) Cross the F_1 males singly with mutant females; (5) If F_1 male chromosomal complement was normal, the F_2 will consist of 1/4 wild-type males, 1/4 mutant males, 1/4 wild-type females and 1/4 mutant females; (6) If F_1 male contained a translocation between the Y-chromosome and the wild-type allele of the mutant locus, the F_2 will consist of 1/2 wild-type males and 1/2 mutant females. This line is retained.

The presence of the chromosomal translocation in the line could be verified through the following types of evidence: (1) Reduced egg hatch; (2) Pseudo-linkage of genetic markers; (3) Cytogenetic studies.

Y-autosome translocations have been produced for the sheep blowfly, *L. cuprina* (Wied.), by Whitten and co-workers [4, 8]. These lines involved the pupal colour mutation 'black' and the eye colour mutation 'white', 'topaz' and 'yellow' which were all autosomal and recessive. Similar translocation lines were developed by silk-worm breeders for autosomal mutations involving egg, larval and pupal colours [10]. It should be noted that the female of the silk-worm is the heterogametic sex whereas the male of the sheep blowfly and other fly species is the heterogametic sex. Y-autosome translocations lines involving autosomal, morphological characters have also been developed for the Mediterranean fruit fly, *Ceratitis capitata* Wied., and the rest of the discussion will deal with this fruit-fly species.

DEVELOPING GENETIC SEXING SYSTEMS WITH MORPHOLOGICAL CHARACTERS FOR THE MEDITERRANEAN FRUIT FLY

The Mediterranean fruit fly, *C. capitata* Wied., is a serious pest of many tropical, subtropical and deciduous fruit trees in various agricultural areas throughout the Mediterranean region, south and central America, Hawaii, Africa and Australia. It has recently invaded California more than once, where there is at present an intensive eradication campaign. The first field experiment with SIRM was carried out on the island of Capri in 1967 [18]. Numerous experiments as well as large-scale field releases have been carried out since then throughout the globe. At present, Mexico-Guatemala and California are the areas for ongoing SIRM programmes for the control of the pest. These programmes rely on the mass-production of flies in highly efficient factories producing millions of flies daily. Naturally, the end product in these factories contains males and females in equal numbers. The females are more than superfluous in the release projects. They also cause damage in the form of 'sterile stings' by attempting to oviposit in the fruit in the field. The lack of proper genetic sexing seems therefore a major obstacle in the expansion of the SIRM of the Mediterranean fruit fly and its adoption by additional countries.

To develop a sound genetic sexing method, some knowledge of the formal genetics of the organism is required. Today, various genetic and entomological laboratories are working to accumulate enough genetic data on the Mediterranean fruit fly. This fly has five pairs of autosomes and a pair of sex chromosomes. The female carries two large sub-telocentric X-chromosomes and the male carries an X-chromosome and a small Y-chromosome [19]. Maleness is determined by the presence of the Y-chromosome (Lifschitz, personal communication). Lifschitz and co-workers state that the integrity of the Y-chromosome is not essential for determining maleness, and even a small piece of it will suffice. They postulated that autosomal genes which have been translocated to the Y-chromosome will show total holandric inheritance.

Some progress has been made in the field of the formal genetics of the medfly. Much more is needed in the future. To date we may present a list of 11 morphological mutants which have been accumulated in six laboratories. Some of these mutants are probably identical (Table II). In addition, seven different biochemical loci have been identified and isolated. The morphological loci are all autosomal. Three linkage groups have been ascertained: Apricot-eye and double-chaetae with 18.6–21.6% recombination in females, orange-eye and white-pupae with 45–46% recombination in females, and niger and esterase-1 with nearly 50% recombination in females.

Two morphological mutants were used to construct medfly lines for genetic sexing. These were the 'dark-pupae' and 'white-pupae' mutants. We have also constructed a sex-limited dimorphic line with the 'apricot-eye' mutation for

TABLE II. MORPHOLOGICAL MUTATIONS OF THE MEDITERRANEAN FRUIT FLY [a]

Symbol	Name and description	Source [b]
ap	apricot-eye, autosomal	Rössler
bl	white-eye, autosomal	Robinson
re [c]	red-eye, autosomal	Lifschitz
or [c]	orange-eye, autosomal	Rössler
ro [c]	rose-eye, autosomal	Robinson
w	white-pupae, autosomal	Rössler
dp	dark-pupae (black), autosomal	Rössler
nig	dark-black pupae, autosomal	Lifschitz
Dp	dark-pupae, autosomal, dominant	Cavicchi
dc [d]	double-chaetae, autosomal, two pairs of lancet-shaped caetae on male's head	Rössler
dc [d]	double-chaetae (as above)	Lifschitz

[a] From the Report of a Consultants Meeting on a Genetic Sexing Mechanism for the Mediterranean Fruit Fly, *Ceratitis capitata*, 24 Dec. 1980, IAEA, Vienna.

[b] Cavicchi, S., Institute of Genetics, University of Bologna, Bologna, Italy.
Lifschitz, E., Dept. of Genetics, INTA, Castelar, Argentina.
Robinson, A.S., Foundation ITAL, 6700AA, Wageningen, The Netherlands.
Rössler, Y., The Israel Cohen Institute for Biological Control, CMBI, 27 Keren Kayemet Le'Israel Street, Rehovot, Israel.

[c,d] Synonyms?

TABLE III. MORTALITY OF IMMATURE STAGES OF THE MEDITERRANEAN FRUIT FLY LINES 69, 69 APRICOT AND 127 [a]

Life stage	Line 69 (%)	69 apricot (%)	Line 127 (%)
Egg	31.0	28.5	36.3
Larvae	57.7	58.5	35.6
Total	67.5	68.7	59.1

[a] Corrected with Abbott's formula: mortality of the normal Mediterranean fruit fly line was assumed as 0%.

experimental purposes. The scheme outlined above was used. Wild-type male were irradiated with 1.2 krad. When 'dark-pupae' was used as the 'sexing character', a total of 86 single-pair crosses resulted in two lines with wild-type males and 'dark-pupae' females (Line 69 and Line 127). Line 69 was further manipulated, and the 'apricot-eye' mutation was inserted, so that the new line '69 apricot' had brown-pupae males and dark-pupae females, and the adults had apricot-coloured eyes. The eye colour could be used as a marker to identify the released males in future release programmes. The three lines had apparently the following genetic makeup:

Line 69	♂♂ T(Y:dp$^+$)/dp	♀♀ dp/dp
69 apricot	♂♂ T(Y:dp$^+$)/dp; ap/ap	♀♀ dp/dp; ap/ap
Line 127	♂♂ T(Y:dp$^+$)/dp	♀♀ dp/dp

As yet we have no cytological proof of the existence of the Y-autosome translocation. We had, however, assumed the veracity of that genotype by the fact that the wild-type allele of 'dark-pupae' was inherited holandrically, and egg hatch as well as larval survival were reduced, as expected for crosses involving heterozygote translocation individuals (Table III).

The attempt to construct a Y-autosome translocation involving the 'white-pupae' locus resulted in a line which consisted of three phenotypes in equal frequencies: Wild-type males, white-pupae females and white-pupae males. The line (line 93) was maintained in our laboratory for 18 generations. Few wild-type females appeared in the sixth generation and their frequency increased until they reached equality with the other three classes. The genetic constitution of that line remained unsolved and is still a puzzle.

An apparent Y-autosome translocation was induced with the 'apricot-eye' locus. The males of that line had wild-type eyes, which had a red colour with blue overtones, and a peculiar narrow shape. The female had apricot-coloured eyes and their shape was normal.

The construction of Y-autosome translocation lines for genetic sexing is a process that may lead eventually to the incorporation of a high genetic load, and to the competitive inferiority of such lines in field releases. The lines are laboratory reared, irradiated, inbred through single-pair crosses and carry morphological mutations with possible pleiotropic effects of a detrimental nature. There is a grave danger that these lines will be highly handicapped. Thus, the construction of such lines has to be accompanied by scrupulous quality control tests which emphasize the sexual competitiveness of the males. Such tests are an integral part of any mass-rearing project in SIRM [20] and much more so when Y-autosome translocations are involved.

We have carried out some preliminary tests with two of our lines. 'Line 69' which had brown pupae males and 'dark-pupae' females, and '69 apricot' which

TABLE IV. COMPETITIVENESS OF STERILIZED, 69 APRICOT MEDFLY MALES

Ratio: S ♂♂ : N ♂♂ : N ♀♀	Day	Egg hatch (%)	'C' value
0 : 1 : 1	1	95.1	–
	4	91.7	–
	10	85.1	–
1 : 1 : 1	1	50.8	0.952
	4	61.0	0.541
	10	63.6	0.363
5 : 1 : 1	1	40.5	0.302
	4	52.9	0.147
10 : 1 : 1	1	58.3	0.068
	4	57.9	0.057

was actually a 'Line 69' derivative with apricot-eyed adults. The results have been published elsewhere [21, 22]. The studies involved mating ability tests of males and females from the '69 apricot' lines through a 'multiple-choice with direct observation' approach [23]. The tests were carried out by placing males and females of the two competing populations (wild-type and '69 apricot'), and recording the types of adults that mated within a predetermined and limited observation period. The results of these tests were compared with results of similar tests that were carried out with normal apricot-eyed adults [24]. Both '69 apricot' and 'apricot-eye' males showed a lower mating ability than the wild-type males. Thus, the Y-autosome translocation by itself did not affect the mating ability of their carriers, and the reduced mating ability could be attributed to the presence of the 'apricot-eye' mutation. In a separate series of tests we showed that the attractivity and inseminating ability of the '69 apricot' males was neither affected by the chromosomal translocation nor by the 'apricot-eye' mutation [21].

In a modified ratio-test, we have evaluated the sexual competitiveness of Line 69 and '69 apricot' males, through progeny analysis of mixed populations, consisting of translocated males, wild-type males and 'dark-pupae' females in various mating ratios [22]. Here also the presence of the chromosomal translocation did not seem to affect the sexual competitiveness of their carriers. The

presence of the 'apricot-eye' mutation did, however, reduce the performance of the males, but not to a level that hindered completely their effectiveness and eventual use in SIRM. The various tests for sexual competitiveness that were carried out with males carrying a chromosomal translocation revealed no distinct detrimental effect on the sexual competitiveness of these males.

The '69 apricot' as well as Line 69 males were semi-sterile due to the heterozygous condition of the chromosomal translocation. Complete sterility was obtained when males were irradiated with 9 krad in the pupal stage, just before emergence. A dose of 7 krad resulted in 99.3–99.7% sterility (measured as egg hatch), and 5 krad caused only 98.2% sterility. The dose of 7 krad was used to sterilize '69 apricot' males, and the mating competitiveness of these males was compared with fertile, wild-type males by methods devised by Hooper [25] (Table IV).

The competitive value 'C' was calculated with the formula

$$C = \frac{N\,Hn - Ho}{S\,Ho - Hs}$$

Hn – Egg hatch of crosses with normal, wild-type males

Ho – Observed egg hatch in the ratio-test

Hs – Residual egg hatch in crosses with sterile males

S – Number of sterile males

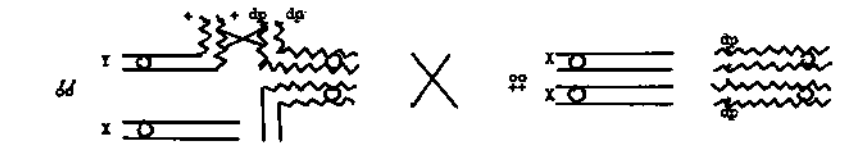
N – Number of fertile males

The results showed that the sterile males were inferior to the wild-type and fertile males. This was not necessarily due to the sterilization effect, but could be attributed to the 'apricot-eye' mutation of the sterile males. The competitiveness of the sterilized males seemed to diminish in time, an aspect that requires further study.

A rather interesting phenomenon was the strong 'minority effect' or the apparent enhancement of the competitiveness of the male class which was in a minority in these tests. When the ratio of sterile to fertile males favoured the sterile males, their competitive value decreased considerably (Table IV).

THE INTEGRITY OF MEDITERRANEAN FRUIT FLY LINES WITH Y-AUTOSOME TRANSLOCATIONS

The Y-autosome translocation line will remain true breeding for the sex-limited trait as long as the 'sexing' locus remains linked to the male's Y-chromosome. The occurrence of genetic recombination (crossing-over) between the



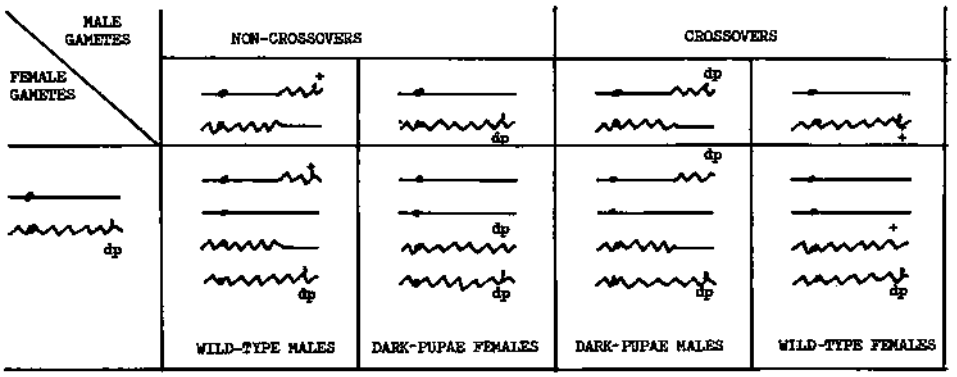


FIG. 1. Progeny of Line 69 of the Mediterranean fruit fly, assuming genetic recombination in males.

'sexing' locus and the translocation point will damage the integrity of the line (Fig. 1). The products of such genetic recombination will shuffle the two alleles of the 'sexing' locus between the males and the females. The line will gradually deteriorate to a state where genetic sexing is no longer feasible. Such a line will eventually reach a steady state with 21.875% wild-type males and 21.875% mutant females, 28.125% mutant males and 28.125% wild-type females, regardless of the rate of genetic recombination in the line. The latter will determine only the speed of the process and the number of generations needed to reach the steady state.

There are various methods to reduce or eliminate the effects of genetic recombination in males on the integrity of the 'genetic sexing' lines. These methods depend on the sex-determination of the insect in question, and on its genetic peculiarities. The target is to suppress the rate of genetic recombination to a very low frequency, or eliminate it altogether. Assuming that genetic recombination does occur in the male, and there is a defined 'maleness factor' on one of the chromosomes, the 'sexing' factor could be placed very close to that factor, so as to eliminate the possibility of chiasmata formation between the maleness and sexing factors. Or, if maleness is determined by the presence of a Y-chromosome, we shall have to produce a translocation that will place the 'sexing factor' as close as possible to the translocation point.

TABLE V. PHENOTYPIC FREQUENCIES IN LINE 69 OF THE MEDITERRANEAN FRUIT FLY

Generation days	Wild-type males (%)	Dark-pupae females (%)	Dark-pupae males (%)	Wild-type females (%)
F_1	42.5	57.5	0	0
F_2	66.7	33.3	0	0
F_3	40.6	53.4	0	0
F_4	41.8	57.5	0	0.7
F_5	50.3	49.6	0.1	0
253	50.0	50.0	0	0
316	49.4	50.6	0	0
385	48.9	50.5	0.4	0.2
451	51.1	47.3	0.8	0.8
645	49.3	44.7	4.4	1.6
760	18.5	50.5	30.2	0.8

TABLE VI. PHENOTYPIC FREQUENCIES IN LINE 127 OF THE MEDITERRANEAN FRUIT FLY

Generation days	Wild-type males (%)	Dark-pupae females (%)	Dark-pupae males (%)	Wild-type females (%)
F_1	39.1	30.5	0	30.4
F_2	64.4	21.9	1.1	12.6
F_3	60.2	36.6	0	3.2
F_6	43.3	56.3	0	0.4
176	50.6	49.4	0	0
265	51.3	47.6	0	1.1
292	51.9	47.3	0	0.8
322	39.2	58.1	0.6	2.1
431	41.8	48.8	7.7	1.7
595	10.9	47.2	40.2	1.7
670	3.1	68.2	26.8	1.9

Another method which is frequently used by geneticists is to insert a chromosomal inversion as a crossing-over suppressor. This method has been used with the mosquito *A. albimanus* Wied. [26], and with *Culex tritaeniorhynchus* Giles [27]. Seawright and co-workers translocated the propoxur resistance locus, which is located on the right arm of the second chromosome, to the Y-chromosome of *A. albimanus* Wied. males. The line consisted of propoxur-resistant males and susceptible females. However, a 27% recombination rate between the propoxur resistant locus and the translocation point made this line unusable. A chromosomal inversion that included the propoxur resistant locus was induced, and that reduced the frequency of genetic recombination to 0.2% in the males. The line In(2R) [T(Y:2R)3] 2 was eventually used in SIRM projects in El Salvador. We should note that among the original translocation lines they also had lines with as low as 4.7% recombination without the inversion.

Obviously, such manipulations are not necessary in insects where genetic recombination is absent or very rare in the heterogametic sex. Morgan [28] stated that genetic recombination did not occur in Drosophila males. That statement was extended by other geneticists to other dipteran males of the sub-order Cyclorrapha, which include fruit flies (Tephritidae), muscoid flies (Muscoidea), blowflies (Calliphoridae) and others. The statement remained unchallenged (with the exception of *Drosophila ananase* Doll.) until the early 1970s. At that time, evidence was accumulated for low frequency but widespread occurrence of genetic recombination in males of diverse Drosophila species [29]. Genetic recombination was also observed in other dipteran males of the sub-order Cyclorrapha such as the housefly *M. domestica* L. [30] and the sheep blowfly *L. cuprina* (Wied.) [31].

Does genetic recombination occur in the medfly males? If so, what would be the effect on the integrity of the medfly lines constructed for genetic sexing? A thorough study of genetic recombination in the medfly males cannot be carried out unless a substantial amount of basic, genetic information has been accumulated. This, unfortunately, is not the case at present. The results presented here have therefore an indicative value only. The studies were initiated after it had been observed that the three Y-autosome translocation lines in the laboratory suffered gradual deterioration and produced increasing numbers of mutant males and wild-type females (Tables V, VI and VII).

Could the deterioration of the Y-autosome lines be ascribed to genetic recombination in the males? To test this assumption we had to show that genetic recombination did occur in the males of the medfly. We made use of the only two linkage groups available. These were the 'apricot-eye' and 'double-chaetae' linkage group and the 'orange-eye' and 'white-pupae' linkage group. Males and females were made heterozygous in coupling or repulsion for these loci and then test crossed (Table VIII). The results showed that genetic recombination between 'apricot-eye' and 'double-chaetae' was either absent or

TABLE VII. PHENOTYPIC FREQUENCIES IN LINE 4 OF THE MEDITERRANEAN FRUIT FLY

Generation days	Wild-type males (%)	Apricot-eye females (%)	Apricot-eye males (%)	Wild-type females (%)
F_1	58.6	41.4	0	0
F_2	44.8	55.2	0	0
F_3	42.1	57.9	0	0
F_4	72.5	27.5	0	0
F_5	58.1	41.9	0	0
163	54.1	45.9	0	0
196	50.3	49.7	0	0
322	47.1	52.6	0.3	0
354	51.3	47.7	0.9	0.1
423	18.1	51.8	30.1	0
460	13.9	54.1	31.9	0.1
507	3.5	44.3	51.2	1.0
518	1.7	49.9	47.5	0.9
546	4.2	45.8	48.6	1.4
Line restarted from parental types				
F_1	40.0	60.0	0	0
41	52.8	47.2	0	0
88	50.6	49.2	0.2	0
172	53.9	43.7	2.4	0
255	32.4	49.2	18.4	0

extremely rare in normal males. The loci 'orange-eye' and 'white-pupae' showed a genetic recombination rate of 0.45% in males.

Test crosses with males heterozygous for 'apricot-eye' and 'double-chaetae', and also carrying a Y-autosome translocation, showed the occurrence of a low frequency of genetic recombination between these two loci (Table VIII). That was true when the 'apricot-eye' mutation and 'double-chaetae' were on the translocated chromosome (Line 4) and on a chromosome which was not involved in the translocation (Line 69) (Table VIII).

TABLE VIII. GENETIC RECOMBINATION IN THE MEDITERRANEAN FRUIT FLY

Cross type	n	Recombination rate (%)
♀♀ ++/ap dc X ♂♂ ap dc/ap dc	7	18.58 ± 4.87
♀♀ + dc/ap + X ♂♂ ap dc/ap dc	2	21.61
♂♂ + +/ap dc X ♀♀ ap dc/ap dc	3	0
♂♂ + dc/ap + X ♀♀ ap dc/ap dc	4	0
♀♀ + +/or w X ♂♂ or w/or w	15	45.89 ± 6.88
♂♂ + +/or w X ♀♀ or w/or w	12	0.45 ± 0.46
♂♂ T(Y:dp$^+$); + +/ap dc X ♀♀ ap dc/ap dc	4	2.27 ± 1.6
♂♂ T(Y:dp$^+$); + dc/ap + X ♀♀ ap dc/ap dc	4	0.16 ± 0.15
♂♂ T(Y:ap$^+$dc$^+$)/ap dc X ♀♀ ap dc/ap dc	4	0.32 ± 0.22

The study is at too early a stage to warrant final conclusions. Nevertheless, some provisional conclusions may be drawn.

(1) Genetic recombination does occur in the medfly males, although at a much lower frequency than in the female.
(2) The presence of a chromosomal translocation increases the incidence of crossing-over in the male, throughout the genom.
(3) The effect of genetic recombination in the males on the integrity of the translocated line depends on the rate of the recombination between the 'sexing' locus and the translocation point.

An enhancing effect of the chromosomal translocation on genetic recombination in males was also observed by Whitten in the sheep blowfly [31]. However, is genetic recombination by itself sufficient to account for the deterioration of the 'genetic sexing' lines?

Foster and co-workers [32] showed that a reversion of the translocation was responsible for the deterioration of the T(Y : 5 : 3) line of the sheep blowfly, which possesed originally a translocation between the Y-chromosome and autosomes 5 and 3. Genetic and cytological studies showed that the line that started as T(Y : 5 : 3) included after a time males with T(Y : 5) translocations and also males with a normal chromosomal complement.

The deterioration of the medfly lines in our laboratory had two peculiarities (Tables V, VI and VII). The deterioration rate was too high to be explained by

genetic recombination in the males only. The deterioration products (wild-type females and mutant males) were unequal in frequency. There seemed to be a fast generation of mutant males, whereas the frequency of wild-type females remained rather low and steady.

It seems that the deterioration process of Y-autosome translocation lines in the medfly is a complicated process and involves more than one mechanism. The future of genetic sexing of that insect, as well as of other insects with a similar pattern, depends on the thorough study of these phenomena, and the formulation of proper counter measures to overcome the problems.

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POSSIBLE COMBINATION OF THE STERILE INSECT TECHNIQUE AND THE TRANSLOCATION METHOD FOR THE CONTROL OF FRUIT FLIES*

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Abstract

POSSIBLE COMBINATION OF THE STERILE INSECT TECHNIQUE AND THE TRANSLOCATION METHOD FOR THE CONTROL OF FRUIT FLIES.

Compared with the Translocation-Method (TM), which is based on y-linked translocations, the Sterile Insect Technique (SIT) has a number of advantages. Because of full fertility, mass rearing is not problematic. The degree of sterility after irradiation is high and therefore an immediate effect of control after adequate releases can be expected. In most fruit fly species females can be sterilized with much lower irradiation doses than males which makes a genetic sexing system not absolutely necessary. On the other hand, males carrying a y-linked translocation can be fully competitive, as they do not have to be irradiated before release. They introduce a genetic load into the field population causing a residual effect after the releases have ceased. The properties of both methods can be combined in the so-called Combi-Fly. In this method a stock of fruit flies is used, carrying a y-linked 3-chromosome double translocation with a degree of inherited sterility of 50 to 66%. This would not cause problems in mass rearing. If the female sterilizing dose of about 4 kR is applied, the resulting sterility in males is as high as 90–95% because there is an additive effect of irradiation- and translocation-induced sterility. These males, when released into the field, could be more competitive, because of the low radiation dose received. The relatively high sterility enables control to be achieved in a period of time comparable with that using completely sterilized insects. All males developing from fertile eggs carry the y-linked translocations and, in addition, in both sexes there will be a considerable amount of new chromosomal rearrangements which increase the genetic load introduced into the field population. The results of the preliminary experiments on the quality and degree of sterility of Combi-Flies are discussed.

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COMPLEX APPROACHES TO INSECT STERILIZATION INCLUDING METABOLIC ACTIVATION AND RADIATION ADJUVANTS*

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Abstract

COMPLEX APPROACHES TO INSECT STERILIZATION INCLUDING METABOLIC ACTIVATION AND RADIATION ADJUVANTS.

A variety of methods other than simple exposure to a radiation source or application of an alkylating agent has been employed to induce insect sterility. These methods include (a) combinations of chemical agents, (b) double-duty pesticides, (c) metabolic activation products and (d) radiosensitizing agents. Respectively these exploratory studies involve (a) two chemicals with different modes of action on the components of reproductive performance, (b) any pesticide that can function also as a chemosterilant, (c) production of a sterilizing metabolite from non-sterilizing precursors and (d) alteration of the cellular environment to enhance the response to ionizing radiations. The new techniques of application devised for almost every agent are indicated, and particular attention is given to the transfer of chemosterilants by contact at mating. Most of the insects considered in the review of literature were pests. In addition the author performed experiments with a braconid wasp which is uniquely suitable for distinguishing between genetic and non-genetic causes of sterility. Topical application of carbamate herbicides was found to decrease egg production during every subsequent period of a treated wasp's life. Dissected ovarioles revealed failure in oocyte vitellogenesis. For the activation of aflotoxin as well as for nitrosation of other compounds, entry via the digestive system was necessary. Two different biochemical mechanisms are involved in the production of these mutagenic metabolites. The respiratory tract plays the important role when gases, vapours, aerosols and fumigants are used in various procedures. Recently it was found that triethylamine together with X-rays decreases fecundity below that obtained from either agent alone. However, to date the dose which can provide competitive males has not been ascertained. A variant of topical application is the transfer of chemosterilant by mating contact. When the various components of fitness were studied for participants and their progenies, tepa was shown to influence many aspects. These included ovarian cytotoxicity, dominant lethality, recessive lethality and sperm inactivation.

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1. INTRODUCTION

The historical development of insect control by induced sterility owed its success to the dominant mutations produced by ionizing radiations or alkylating chemicals [1]. Radiation exposures are a simple matter of proximity to a source. Chemosterilants were applied by spraying, dipping, dusting or contact with an applied droplet or surface residue [2]. Old and new agents in combination and variations in application will be reviewed here along with some results from the author's laboratory. In some topics, space limitations have prohibited direct reference to all related papers. The references given for papers cited must serve as a more comprehensive guide to the literature.

2. HISTORICAL AND EXPERIMENTAL INFORMATION

2.1. Combinations of Chemical Agents

Successful chemosterilization can be accomplished by sequential treatment with two agents that have different modes of action. For example, an alkylating agent, busulfan, in the diet of boll weevils followed by a dip in the chitin inhibitor Dimilin® produced successful sterilization [3]. Busulfan alone allowed the deposit of some fertile eggs but the Dimilin® interfered with their hatching. A special device to expedite dipping was developed in the course of this investigation.

An alternate way to apply chemical agents is by fumigation. Borkovec et al. [4] used a fumigator with a rotating bisazir-treated paddle. Ninety minutes fumigation with the alkylating aziridinyl compound was followed by a 5 minute dip in penfluron, an analog of Dimilin®. Both sexes were sterilized by this technique for which the equipment was scaled up to accomodate 200 000 weevils at a time.

A variation of the fumigation technique is to use reduced pressure in the exposure chamber. This is known as hyperbarometric or vacuum fumigation. An advantage is the short treatment time. Weevils can be sterilized in 1 to 5 minutes but their cuticle must be hard. Conventional fumigation can be applied immediately after emergence while the cuticle is soft; also size of the exposure chamber is no limitation.

For initial trials, hempa and a volatile analogue to busulfan (isopropyl methyl sulfonate) were employed [5]. A more definitive experiment run at a lower temperature combined vacuum fumigation using hempa with 4 days of feeding on a diet containing 0.1% busulfan [6]. The combination treatment accomplished what neither agent could do alone in sublethal doses. Both males and females were sterilized by this procedure.

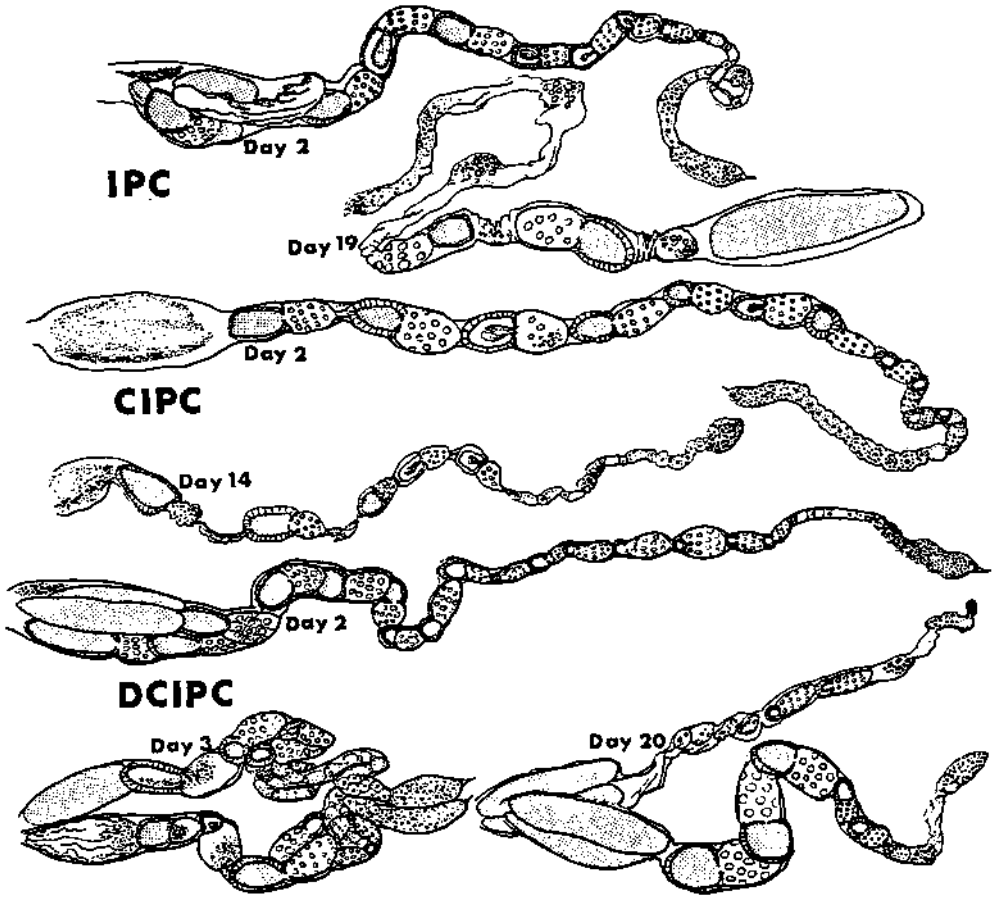

FIG.1. Damage to oogenesis in ovarioles from Bracon *treated topically with a droplet of one of three carbamate herbicides.*

2.2. Double-Duty Agents

A possible but still unattained goal is the discovery of an agent which can control insects as well as unwanted plants. Here are considered three carbamate herbicides that interfere with Bracon reproduction.

Figure 1 shows degenerating units in the oogenetic sequence of braconid females treated topically with either IPC (isopropyl carbanilate), or CIPC (isopropyl m chlorocarbanilate) or DCIPC (isopropyl 3,4-dichlorocarbanilate). Note the 'bulb' type

oocyte resorption on Day 2 for IPC and CIPC. For DCIPC, deterioration became evident on Day 3. Ovarioles dissected out of treated females during the remaining 3 weeks of life showed gaps in the developmental sequence. These were correlated with the poor egg production by females receiving a sublethal dose. The agents are known to be mitotic poisons, but in addition they cause early depletion of the fat body needed to supply vitellogenic protein. A similar somatic tissue response was noted in wasps exposed to carbaryl insecticide [7]. Egg hatchability was not reduced drastically.

A different class of compounds, the s-triazines which include the herbicide simazine, provides another example. When fed to house flies, various intermediates obtained in the synthesis of substituted melamines decrease the proportion hatching and pupating.[1] One of them, ipazine, altered oviposition.

2.3. Metabolic Activation

A recent concern of genetic toxicologists has been the mutagenic metabolites resulting from the action of biochemical systems upon inactive parent compounds. In both types of conversions which we have investigated, braconid wasps were able to accomplish the chemical change via the digestive tract. Our criterion of damage was alteration in reproductive performance.

Table I provides a comparison of results from three modes of application of aflotoxin B_1. Impressive decreases in egg production and hatchability were seen only when the two higher doses were fed. Also this route of entry provided an appreciable proportion of stage 1 deaths, expected from any agent that can induce chromosome aberrations. The data shown are taken from an initial study by L. H. Valcovic at Auburn University. Subsequently we repeated the experiment at N.C. State University and obtained slightly higher values because of higher control hatchability. Nevertheless egg production and hatchability stayed at low values for the remaining life of the treated females. With control hatchability above 90%, eggs derived from transitional cells showed only 27.4 $\pm$ 1.1% hatched, and those from oogonia showed 33.4 $\pm$ 2.5% hatched after the females ingested one meal containing 10 ppm aflotoxin. Near zero hatchability resulted from the 100 ppm meal. In later life of the injected females, the hatchability of the eggs laid approached control levels. Aflotoxin can cause other types of damage to insects. Fed to larvae of three different pests of maize, aflotoxin B_1 or G_1 reduced larval weight and increased mortality [8]. This recent paper cites most of the literature reporting reduced viability of immature stages from most of the insect orders. Decreased fecundity and fertility has

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TABLE I. A COMPARISON OF THE REPRODUCTIVE PERFORMANCE OF SAMPLES OF *Bracon* RECEIVING 0.5 MICROLITERS OF AFLATOXIN B_1 BY THREE DIFFERENT ROUTES

Treatment	Total eggs	Oocyte stage when treated	
		Hatchability (%)	Stage 1 deaths (%)
Control	208.2	71.9 ± 2.6	0.01
Feeding			
100 ppm	77.4	5.7 ± 1.4	0.45
10 ppm	125.4	17.4 ± 1.7	0.35
1 ppm	210.1	77.6 ± 2.4	0.22
Injection			
100 ppm	182.9	69.6 ± 3.16	0.02
10 ppm	144.7	61.2 ± 3.1	0.03
1 ppm	190.1	76.1 ± 3.2	0.00
Topical			
100 ppm	135.4	73.7 ± 3.2	0.12
10 ppm	178.7	75.9 ± 2.8	0.12
1 ppm	159.4	75.0 ± 3.0	0.04

also been noted in adult weevils fed on aflotoxic corn meal [9]. This suggests action as a natural control agent for some insects [10], and provides motivation for testing products from other cultured fungi. However, since mediation by the gut wall is necessary to convert aflotoxin B_1 to the toxic 2,3-epoxide metabolite, ingestion tests are important.

A nitrosation experiment identified a second example in which the effective route of entry for obtaining a sterilant is the alimentary canal. Ethylurea (EU) and $NaNO_2$ fed simultaneously to female braconid wasps caused a decrease in oocyte hatchability and interfered with the differentiation of oogonia into oocytes [11]. No change in fecundity and fertility resulted when the same combination of compounds was injected into the wasp hemocoel. However, the important factor in this case is the pH of the insect's gut. Indeed in an acid pH sodium nitrite can convert a variety of carbamate compounds into a highly mutagenic nitrosocarbamate product. The broad implications of nitrosation are emerging in research performed by H.W. Dorough and associates at the University of Kentucky, but not every species of insect will provide optimum conditions for the chemical conversion.

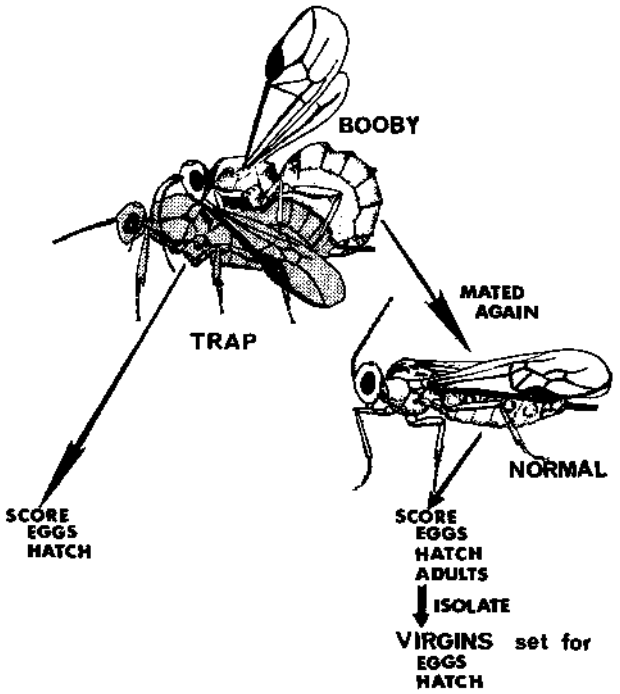

FIG.2. Diagram of steps in a braconid experiment which tests the genetic consequences of chemosterilant transferred by mating contact. The male 'booby' carries the sterilant from the treated female to a second mate. Dominant lethals appear in her offspring and recessive lethals express themselves in the parthenogenetically produced offspring of her daughters.

2.4. Chemosterilant Transfer by Mating Contact

This approach can be employed with a wide variety of agents, but has not been fully exploited. A suggestion by E.F. Knipling [12] stimulated the forerunner of several types of experiments. Morgan [13] 'booby trapped' female house flies by cementing a chamois pad to the abdomen. This was loaded with metepa before the females were released into cages of mature males which were used subsequently in test matings. At the highest dose used, only half the matings approached 100% sterility. Concurrently a simpler method was tested on sheep blowflies [14] when the chlorinated hydrocarbon dieldrin was applied directly to the abdomens of females from a resistant strain. In turn the insecticide was transmitted in lethal dose to up to 100 males by each carrier female. Meanwhile ^{14}C-labelled tepa, originally injected into male houseflies, was detected in the bodies of females [15].

TABLE II. THE FECUNDITY AND FERTILITY OF THE TRAPS AND OF FEMALES MATED TO BOOBIES

Mean egg production and hatchability with standard errors

Samples	0.1 M tepa	(days 1–4)[a]	0.5 M tepa	(days 1–5)[a]
	Eggs	Hatch (%)	Eggs	Hatch (%)
Control	16.1 ± 0.7	96.4 ± 1.9	–	–
Traps	2.6 ± 0.7	0	0	–
Mated at 4 h	12.2 ± 0.9	30.8 ± 3.2	14.2 ± 0.7	34.9 ± 2.3
Mated at 24 h	12.5 ± 0.7	61.7 ± 4.5	16.4 ± 0.8	67.2 ± 3.2
Second mating control	12.7 ± 0.8	92.0 ± 2.6	15.8 ± 1.1	96.5 ± 1.4

[a] Tested during young adulthood subsequent to treatment.

The results with diverse agents encouraged a group in Prague to develop potent synthetic juvenile hormone analogues that could be spread by mating contact [16, 17]. The males of Pyrrhocoris apterus L. (Hemiptera) tolerate large amounts of JH analogues and induced sterility was especially pronounced when females mated repeatedly with treated males. Dimilin®, an inhibitor of chitin synthesis, is still another compound which can be passed along from males to females and decreases egg hatchability [18].

Figure 2 diagrams an experiment designed to give a more analytical test of tepa transfer from two dose levels applied topically to abdomens of females. The lower dose, 0.01 M, allowed traps to lay a few unhatchable eggs. No eggs were laid after females received 0.001 ml of the 0.5M tepa. Post-treatment lifespan was also dose related: 7 days and 5 days respectively. On the other hand, comparisons of the second and fourth columns of Table II reveal no significant difference between the hatchability of eggs laid by females mated to the boobies that transferred tepa from the two levels of treatment. Within each dose hatchability was decreased more by sperm acquired at mating at the fourth hour than when mating was delayed for 24 hours. Fecundity and fertility were still low when tested 2 to 3 weeks later during the senility of the females.

Additional information was provided by the sex ratios of adult progeny. All control matings produced 2/3 biparental offspring but in the experimental group significant decrease in biparental females was induced in the progeny of observed matings.

Tepa-induced sperm inactivation and dominant lethality was first reported in the wasps exposed to coated surfaces [19]. The booby trap results resemble those obtained from a 5-minute exposure to a residual deposit.

Because of the parthenogenetic production of males the rate of recessive mutation can be determined for all loci in the progeny of virgin females. Virgins fathered by boobies treated with 0.1 M tepa produced sons with 6.6% and 4.3% recessive lethals respectively for the mating transfers at the 4th and 24th hours. This exceeds the percentage of recessives induced by a 3-minute exposure of males to a surface coated with 2.12×10^6 mg tepa/mm^2 [20]. It resembles the average yield from a 5-minute exposure.

2.5. Radiosensitizing Agents

Radiosensitization occurs if something increases the cellular response to irradiation by altering the internal environment in cells. For cancer therapy, agents have been developed which promote radiation destruction of the anoxic regions of mammalian tumors for which the vascular system has been impaired. Effective agents fall into three main classes [21, 22].

Type 1. Nitric oxide and nitroxyl compounds which, like oxygen, are expected to have the chemical properties needed for the fixation of damage. Type 2. N-ethylmaleimide and other SH binding agents. Type 3. Electron-affinic compounds, usually nitroaromatic structures, which can capture electrons and promote migration to increase the number of negative radical ions at the intracellular target.

Ordinarily the tracheal supply of air is adequate to sensitize insect tissues, but anoxia can occur from overcrowding in air-tight containers for prolonged periods [23]. This tends to nullify the sterilizing effects of radiation. Here the alternative is aeration, or even hyperbaric oxygen treatment. Using an agent which renders cells only as sensitive as they are when irradiated in the presence of oxygen is not justified. Furthermore, for optimum effect the sensitizers developed for human therapy must be injected, not a feasible technique for mass treatment of insects.

On the other hand, changing the atmosphere for a radiation exposure is a simple matter if the holding chamber is equipped with stopcocks. Thus when irradiation in air decreased the sexual competitiveness of male medflies, a less drastic situation for somatic tissues was sought. These studies showed that irradiation of pupae in nitrogen produced sterile males more sexually competitive and more capable of producing sex pheromones than those irradiated in air [24], although the krad doses used were still adequate to cause the desired damage in sperm.

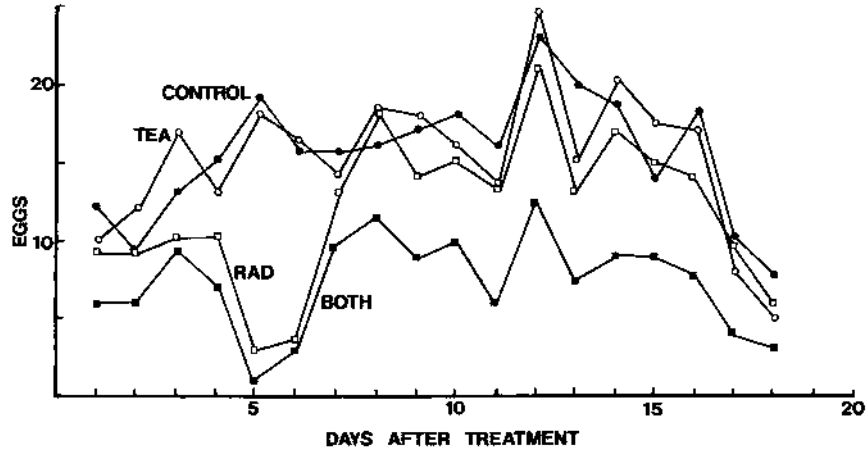

FIG.3. Average daily egg production per Bracon *female. TEA = exposed to fumes from triethylamine, RAD = irradiated with acute dose of 4000 rad of ^{60}Co gamma rays, BOTH = irradiated while anesthetized with TEA. (1 rad = 1.00 × 10^{-2} Gy.)*

Earlier, LaChance [25] demonstrated the protective effect of either N_2 or CO_2 on all three components of female sterility in irradiated screw-worm pupae. On the other hand, when the pupae were irradiated in a 50:50 mixture of CO_2 and air, damage to adult reproductive performance was significantly greater than that induced by the same radiation dose delivered in air. Only 80% of the gamma dose delivered in air was required to reduce egg production to 4% of control values and hatchability to zero. If a 45-minute pretreatment in the gas mixture was included, damage to oogenesis exceeded that obtained by irradiation in pure oxygen. Enhanced radiation damage also occurs in Dipteran spermatogenesis when O_2 as well as CO_2 is present [26]. Two complex mechanisms may be involved. CO_2 inhibition of respiration may make more of the O_2 available for forming the peroxyls that function in the indirect action of radiations, and/or radiation repair systems may be inhibited.

For Drosophila, there is a considerable literature on modifying the spermatogenic stage-dependent differences in radiosensitivity. Many of these studies which were primarily concerned with specific cell types employed respiratory inhibitors [27]. Although interesting from the standpoint of theoretical radiobiology, their scope exceeds what can be included here.

Vapors, aerosols and fumigants are equally suitable for exploiting the tracheal route of entry. Recently we have identified an agent giving off vapors that increase the effectiveness of radiation doses to female wasps. It is triethylamine

TABLE III. THE HATCHABILITY OF EGGS LAID AFTER TREATMENT WITH TRIETHYLAMINE, 4000 rad OF GAMMA RAYS, OR BOTH
(Means ± S.E. for the eggs from 15 females in each treatment group)

Group	Days after treatment[a]			
	1–5	6–10	11–15	16–20
Control	95.6 ± 1.2	89.4 ± 1.4	75.1 ± 1.5	59.9 ± 3.0
TEA	91.7 ± 2.1	89.9 ± 1.9	68.9 ± 4.6	49.3 ± 3.2
Radiation	71.4 ± 4.6	43.9 ± 5.3	52.8 ± 4.4	43.3 ± 4.0
Both	85.6 ± 4.2	50.2 ± 6.8	49.3 ± 4.3	55.4 ± 5.1

[a] The 5-day periods correspond to youth, adulthood, middle age and senility of the females. The eggs laid were derived from oocytes, transitional cells and oogonia present at the time of treatment.

(TEA) diluted in acetone. Figure 3 shows that TEA in combination with radiation decreased egg production below that of radiation alone for every day from youth to senility. A more concentrated solution in combination with the same dose of radiation destroyed all of the oogenetic cells except for those providing a few eggs on days 5 and 8. The hatchability of the eggs from Figure 3 is summarized on Table III, by periods based on the cytological condition at treatment and age of the mothers at time of deposit. Evidently for this criterion most of the damage is radiation induced.

Only one dose level has been tested with males. A one-minute exposure in a shell vial containing 0.5 ml of a 50:1 dilution of TEA anesthetized the males for an hour. Subsequently they were tested every hour for the characteristic recognition response to a female's presence. Finally at the seventh hour one mated. The other six males were stored over night without females and tested again the next morning. At noon, after 4 hours without showing interest in females, each male was paired with a female in a vial provisioned with host caterpillars. In comparison with control vials, very few biparental progeny were obtained. TEA exposures less damaging to the male behavior will have to be established if radiation sterilization could be successful.

3. CONCLUDING REMARKS

If the approach which succeeded so well with the screw-worm fly had proven universally applicable, the investigations reviewed

here would not have been pursued. In particular the dose-limiting sensitivity of somatic tissues in other species has contributed to a study of modified sterilization techniques. Often these have involved treatments supplemental to simple irradiation or alkylation. At the same time, the philosophy of control programs has shifted from eradication toward pest management, which attempts to integrate man-imposed actions with those of the ecosystem in lowering pest populations. Conceivable methods which kill most but not all offspring merit consideration for promoting a trend toward extinction. However, this would mean that steps taken to induce sterility would form part of a strategic plan, rather than to bear the entire burden of control. Incidentally it is interesting to note that the screw-worm eradication in Florida was facilitated by unusually cold weather which reduced the overwintering area [1].

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QUALITY CONTROL OF FRUIT FLIES

Session 6

DETERMINACION DE LA DOSIS OPTIMA DE IRRADIACION RELATIVA A LA COMPETITIVIDAD DEL MACHO ESTERIL DE *Anastrepha ludens* (Loew); SU ATRACCION A TRAMPAS DE COLOR Y AL ATRAYENTE SEXUAL

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Abstract–Resumen

DETERMINATION OF OPTIMUM IRRADIATION DOSES IN RESPECT OF THE COMPETITIVENESS OF STERILE MALES OF *Anastrepha ludens* (Loew); ATTRACTION TO COLOUR TRAPS AND TO SEXUAL ATTRACTANTS.

Fruit cultivation is of great socio-economic importance in Mexico and covers an area of 650 000 ha. One of the most important pests is the Mexican fruit fly *Anastrepha ludens* (Loew). Its study is of topical importance since the Directorate General of Plant Health (SARH) and the United States Department of Agriculture have embarked on a joint control and eradication programme through APHIS. The results relate mainly to the effect of gamma radiation on sexual competitiveness and behaviour. The radiation was applied in the pupal phase three days before emergence, and sterility and sexual competitiveness were studied in the laboratory and in the field. The following conclusions were reached: (a) The males showed 90% sterility at 4 krad and practically 100% at 6 krad. A dose between 3 and 5 krad may be sufficient to obtain a high degree of sterility with a minimum of effect on sexual competitiveness; (b) 'solar' yellow increases the efficiency of traps by a factor of three to five; (c) the live-male bait attracts the greatest number of flies of both sexes, and the male was found to secrete a sex pheromone and an aggregation pheromone; (d) in all cases a significantly higher number of normal than irradiated flies were captured in the traps. This at least shows that the dose of 6 krad affects the adults' flying capacity or perception of attractants. Colouring also reduced the number of flies attracted.

DETERMINACION DE LA DOSIS OPTIMA DE IRRADIACION RELATIVA A LA COMPETITIVIDAD DEL MACHO ESTERIL DE *Anastrepha ludens* (Loew); SU ATRACCION A TRAMPAS DE COLOR Y AL ATRAYENTE SEXUAL.

La fruticultura en México es de gran importancia socio-económica, siendo el area de cultivo de unas 650 000 ha. Una de las plagas más importantes es la mosca mexicana de la fruta, *Anastrepha ludens* (Loew). Su estudio reviste importancia actual, ya que la Dirección General de Sanidad Vegetal (SARH) y el Departamento de Agricultura de los Estados Unidos han iniciado, a través de APHIS, un programa conjunto de manejo o erradicación. Los resultados se relacionan principalmente con radiación gamma en su competencia sexual y comportamiento. Esta se llevó a cabo en la fase de pupa, tres días antes de la emergencia,

y los trabajos sobre esterilidad y competencia sexual se hicieron en los laboratorios y en el campo. Las conclusiones a las que se ha llegado son: a) Los machos presentaron un 90% de esterilidad con 4 krad y prácticamente el 100% con 6 krad. Las dosis comprendidas entre 3 y 5 krad pueden ser suficientes para obtener una alta esterilidad afectando en un mínimo la competencia sexual; b) El color amarillo solar aumenta la eficiencia de las trampas de tres a cinco veces; c) El cebo a base de machos vivos atrajo el mayor número de moscas de ambos sexos, comprobándose que el macho emite una feromona sexual y otra de agregación; d) En todos los casos las trampas capturaron un número significativamente más alto de moscas normales que de irradiadas; esto indica cuando menos que la dosis de 6 krad afecta a los adultos en su capacidad de vuelo o en la percepción de los atrayentes. También el marcaje con colorantes redujo la cantidad de moscas atraídas.

1. INTRODUCCION

La fruticultura en México tiene un gran valor socio-económico. En efecto, refiriéndonos únicamente al naranjo, toronja, mango y guayaba, la superficie de cultivo es de 210 000 ha, con un valor económico de hasta 1751 millones de pesos mexicanos en 1976 [1] y mucho mayor actualmente.

Cada año, la mosca mexicana de la fruta *Anastrepha ludens* (Loew) daña las cuatro especies de frutales mencionadas, ocasionando pérdidas [2] de alrededor del 10%, lo que implica un valor económico actual de 300 millones de pesos aproximadamente. Asimismo, esta plaga ha frenado el desarrollo frutícola en México y Centroamérica.

Además de las pérdidas directas por el daño del insecto, existen otros factores tales como los costos por muestreo de la plaga y las medidas de supresión, tanto en las huertas como en la fruta para exportación, lo que grava considerablemente la economía de los productores.

Aunque existen diversos estudios sobre aspectos bioecológicos y de control de la especie, hasta la fecha no se ha logrado una reducción satisfactoria de las pérdidas ocasionadas por la plaga.

Tomando en cuenta lo anterior, se consideró necesario llevar a cabo diversos estudios tanto para obtener información que afine los conocimientos existentes como para lograr la implantación de técnicas nuevas que nos sitúen en mejor posición para la solución de este tipo de problemas.

Los objetivos de este estudio fueron: a) determinación de la dosis más adecuada de irradiación; b) competitividad del macho estéril; c) respuesta de los adultos irradiados y normales al color de trampa y a varios atrayentes sexuales.

Recientemente, los resultados de esta y otras investigaciones en el futuro están tomando una importancia especial, ya que la Dirección General de Sanidad Vegetal de nuestro país y la APHIS-PPQP del Departamento de Agricultura de los EE.UU. tienen planes para iniciar un programa de manejo o erradicación de *A. ludens* cuando menos en el noreste de México y suroeste de los Estados Unidos.

Estos estudios se llevaron a cabo en el recinto del Instituto Tecnológico y en su campo agrícola experimental de Apodaca, durante 1978 y 1979.

2. MATERIALES Y METODOS

Todo el material biológico de moscas para los diferentes estudios, tanto normal como irradiado, fue proporcionado por el laboratorio de cría masiva y de esterilización de la mosca mexicana de la fruta en Monterrey, la APHIS-PPQP del Departamento de Agricultura de los EE.UU. y la Dirección General de Sanidad Vegetal de la Secretaría de Agricultura y Recursos Hidráulicos.

El material irradiado se trató en estado de pupa, de nueve días de edad y tres días antes de la emergencia del adulto en un irradiador GAMMACELL 220 a base de ^{60}Co [3].

El material biológico se colocó en jaulas de emergencia de armazón de madera de 50 X 27 X 27 cm de largo, ancho y alto respectivamente, forradas con tela metálica tipo mosquitero. El agua se suministró en frascos de 150 cm^3 con tapa perforada y mechas de algodón; el alimento consistente en 3 : 1 partes de azúcar y proteína hidrolizada se puso en vasos de plástico.

El sexado se hizo en adultos de dos días de edad, utilizando un motor de vacío y un frasco colector de 900 ml, con una capa gruesa de hule espuma en el fondo para amortiguar el contacto de los insectos con el frasco.

El marcado de los insectos para las pruebas sobre atracción al color amarillo de trampas y atracción sexual se hizo con el pigmento Calco Blue® en la proporción de 3 g por litro de pupa. Las pupas se colocaron en una bolsa de plástico, depositando el pigmento sobre ellas y luego con movimiento continuo se logró la impregnación uniforme del polvo. A continuación se pusieron las pupas en recipientes cilíndricos de medio litro, colocándolos en el interior de la jaula de emergencia.

Las condiciones de temperatura y humedad en el laboratorio se registraron con un higrotermógrafo.

2.1. Esterilidad natural con respecto a la edad de las moscas

Para esto, se colocaron 100 hembras y 100 machos normales en jaulas para cópula, con agua y alimento y un dispositivo para oviposición. Las jaulas fueron construidas con dos lados de madera y los restantes de tela metálica tipo mosquitero, de 22 X 22 X 22 cm de largo, ancho y alto respectivamente.

Desde el inicio de la oviposición, diariamente se tomó una muestra de 100 huevecillos, que se colocaron en hileras sobre papel filtro negro húmedo en cajas de Petri, poniendo además un algodón pequeño empapado con una

solución de benzoato de sodio al 0,7% para evitar fungosis; se taparon y se sellaron las cajas con cinta adhesiva y se pusieron en una incubadora a 21 a 25°C y a 70% de humedad relativa durante 5 días. Al sexto día se observó todo el material en microscopio estereoscópico, contando los huevecillos eclosionados y los infértiles.

2.2. Determinación de la dosis óptima de irradiación

Para este estudio se sexaron moscas recién emergidas, antes de la cópula, poniendo 25 machos estériles y 25 hembras normales (cruzas 1 : 1) en jaulitas de armazón de madera de 15 × 15 × 15 cm, cubiertas en cuatro de sus lados con "triplay" y los restantes con tela de plástico tipo mosquitero; el agua y el alimento se dispusieron de una forma similar a la de las pruebas anteriores. Cada jaulita formó parte de una serie de cuatro incorporadas a un bastidor de madera. Como dispositivo para oviposición se puso una tela parafinada de color anaranjado, moldeada a manera de media naranja para que las hembras ovipositaran. Para evitar la deshidratación de los huevecillos, se mantuvo la humedad poniendo papel absorbente humedecido en el hueco del moldeado y se cubrió con una caja de Petri. Cada prueba constó de 9 tratamientos (0,5, 1,0, 2,0, 4,0, 6,0, 8,0, 10,0, 12,0 krad y el testigo) y cuatro repeticiones.[1] Las muestras para estudio se tomaron dos días después de iniciada la oviposición; se recolectaron 100 huevecillos por repetición para hacer un total de 400 por tratamiento. La incubación y los recuentos de fertilidad se hicieron de una forma similar a la de las pruebas anteriores.

Se hizó una prueba similar 20 días después de iniciada la oviposición para detectar una posible recuperación en la fertilidad.

Para conocer el efecto de la irradiación en las hembras, se hicieron dos pruebas cruzando hembras estériles y machos normales (cruzas 1 : 1) haciendo la evaluación a los 3 y 20 días de iniciada la oviposición.

2.3. Competitividad del macho estéril

Al no contar con información suficiente para esta especie, se consideró necesario mayor investigación para obtener resultados aplicables a programas basados en la técnica del insecto estéril.

Para estos estudios, se sexaron moscas recién emergidas antes de copular, colocando 90 hembras y 90 machos estériles con 10 hembras y 10 machos normales del laboratorio en las jaulas ya descritas para cópula y oviposición, en la relación de 9 : 9 × 1 : 1 y en las dosis utilizadas (0,5, 1,0, 2,0, 4,0, 6,0, 8,0, 10,0, 12,0 krad y testigo).

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[1] 1 rad = 1.00×10^{-2} Gy.

Se llevaron a cabo otros experimentos colocando 190 hembras y 190 machos estériles con 10 machos y 10 hembras normales de laboratorio, en una relación de 19 : 19 X 1 : 1.

Los experimentos para ambas relaciones se hicieron con nueve tratamientos y cuatro repeticiones.

Los cuidados de los adultos, la toma de muestras de huevecillos y su manejo en la incubadora y el recuento de fertilidad se hizo de forma similar a la de la determinación de dosis.

2.4. Influencia del color de la trampa en la captura de adultos

Tomando en cuenta que diversos investigadores, entre ellos Prokopy [4], Remund y Boller [5] y Leos [6], reportan para otras especies de la misma familia diversos tipos de trampas que han resultado más eficientes que la McPhail, se consideró necesario llevar a cabo una serie de pruebas evaluando básicamente el poder atrayente de trampas rectangulares de cartón de color amarillo solar (pintura COMEX 18–02) comparándolas con trampas transparentes de mica.

Las dimensiones de las trampas fueron de 30 X 20 X 0,2 cm de largo, ancho y grueso respectivamente. Para facilitar su manejo y prolongar su duración durante las pruebas, se cubrieron con una bolsa de plástico transparente que contenía una capa delgada y uniforme de una grasa especial Stikem®, siguiendo en parte el procedimiento de Kaloostian [7] y Leos [6].

Las pruebas tuvieron lugar en un huerto de naranjo plantado a 8,0 m en sistema "tres bolillo" y se utilizó un diseño de observaciones apareadas colocando en forma alternada 12 trampas amarillas y 12 blancas colgadas a 1,60 m de altura en la parte exterior del árbol y en dirección norte-sur. Una vez instaladas las trampas, se liberaban las moscas poniendo 1000 insectos de 2 días y 1000 de 12 días en la parte central de cada cuatro árboles en nueve sitios, liberando pues un total de 18 000 moscas por prueba; de esta forma se logró una distribución uniforme de la población y todas las trampas tuvieron la misma probabilidad de capturar moscas.

Todos los días, a las 6 de la mañana, se hicieron recuentos de hembras y machos de todas las moscas atrapadas, separándolas con pinzas y continuando esta operación hasta que la población dejó de caer en las trampas.

Las condiciones ambientales (temperatura y humedad relativa) durante estas pruebas y las de atracción sexual se registraron en la estación meteorológica del campo experimental.

2.5. Atracción sexual en el campo

Hasta la fecha, en actividades de campaña, detección, dinámica y supresión de la mosca mexicana de la fruta se han utilizado casi en forma exclusiva

atrayentes de tipo alimenticio cuya composición básica es una proteína hidrolizada; solo en forma experimental se han hecho pruebas preliminares con esencias de algunas plantas y atracción sexual [8, 9, 6].

Se consideró conveniente, pues, llevar a cabo algunos estudios para obtener mayor información, que pudiera ser utilizada en proyectos nuevos de investigación y en programas de supresión a nivel comercial.

Debido a que para estas pruebas se usaron como atrayentes, entre otros, extractos tanto de machos como de hembras, se utilizó para su obtención un procedimiento similar al empleado por Fletcher et al. [10] para el gusano tornillo *Cochliomyia hominivorax* (Coquillet) conocido como condensación de vapores por frío para extracción de feromonas. Para nuestra investigación se dispuso de un aparato[2] compuesto esencialmente de tres partes: una cámara de confinación de insectos, una cámara de condensación de vapores y un extractor eléctrico.

2.5.1. Pruebas en el campo

Inicialmente se llevaron a cabo dos pruebas utilizando el diseño de observaciones apareadas con dos tratamientos y 12 repeticiones.

Uno de los tratamientos consistió en trampas transparentes de mica embadurnadas con pegamento Stikem® únicamente. El segundo tratamiento incluyó además de la trampa pegajosa una jaulita de 4 × 4 × 10 cm con 25 machos normales vivos, usados como cebo, colocada en la parte central de la trampa grande. Las trampas se dispusieron bajo los árboles a 1,60 m de altura y en forma alternada de modo que, tanto en línea como a los lados, quedara una trampa con atrayente frente a una sin él.

Una vez instaladas las trampas, se liberaron las moscas poniendo 1000 insectos machos normales y 1000 irradiados en el centro de cada cuatro trampas hasta completar nueve sitios de liberación con un total de 18 000 moscas por prueba.

En la primera prueba se marcaron las moscas irradiadas y en la segunda las normales para detectar si el marcador afectaba a las moscas.

Diariamente, durante 7 días, se hicieron recuentos a las 6 de la mañana, anotando las moscas capturadas por cada trampa.

Finalmente, se hicieron dos experimentos para evaluar el efecto de varios atrayentes para machos y hembras, utilizando para ello el diseño de bloques al azar con seis tratamientos y cuatro repeticiones.

Los tratamientos fueron: 1) machos vivos; 2) Torula (proteína hidrolizada); 3) extracto de hembras; 4) testigo (trampa con grasa pegajosa únicamente); 5) extracto de machos; 6) hembras vivas.

[2] Diseño y construcción de L.E. Garza Blanc, alumno del Progr. de Grad. Agric. del Instituto Tecnológico de Monterrey, Nuevo León.

En los tratamientos en los que se utilizaron machos o hembras vivas como cebo, se pusieron las moscas en jaulitas de 4 X 4 X 10 cm.

Para la Torula se usaron frasquitos de 150 cm^3, poniendo 100 cm^3 de agua y una pastilla dos días antes para lograr una adecuada fermentación desde el principio del experimento.

Para los extractos de moscas tanto de machos como de hembras, se usaron los mismos frascos de 150 cm^3, a los cuales se les introdujo un algodón que, diariamente a las 6 de la mañana y a las 5 de la tarde, se empapó con 10 cm^3 del extracto objeto de prueba.

Todos los dispositivos se colocaron en la parte central de las trampas, sujetándolas con alambre delgado amarrado a los ganchos de alambre grueso utilizados para colgar las trampas en los árboles de naranjo.

La liberación de las moscas y los recuentos de capturas se hicieron de forma similar a la de los dos experimentos anteriores.

En el penúltimo experimento se liberaron machos y en el último hembras para evaluar el poder atrayente de los diferentes tratamientos (atrayentes) para ambos sexos.

3. RESULTADOS Y DISCUSION

A continuación se presentan los resultados y la discusión de estos estudios hechos en laboratorio y campo.

3.1. Esterilidad natural con respecto a la edad de las moscas

Los valores promedio de esterilidad en cuatro pruebas hechas a 21–26°C y una humedad relativa de 60–70% se presentan en la Fig.1. La tendencia de la curva muestra claramente que el menor índice de esterilidad de *A. ludens* se produce durante la primera semana después de iniciada la oviposición; después, la esterilidad se incrementa rápidamente, alcanzando su máximo nivel de alrededor del 90% a fines de la segunda semana. Este resultado debe considerarse al hacer estudios sobre irradiación. Según una información muy reciente, la calidad de las moscas de la unidad de cría era algo baja, lo que explicaría este resultado.

3.2. Determinación de la dosis más adecuada de irradiación de pupas

Los resultados de las pruebas efectuadas a 21–26°C de temperatura y 65–70% de humedad relativa se presentan a continuación.

La esterilidad de los machos estériles provenientes de pupas sometidas a diferentes dosis de irradiación, cruzados con hembras normales, se presenta en

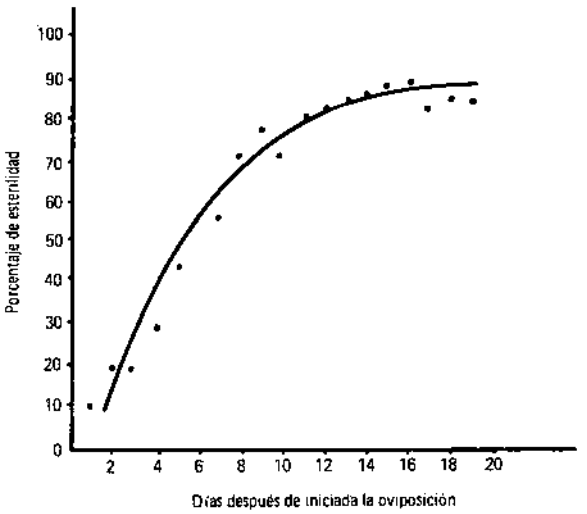

FIG.1. Fluctuación de la esterilidad natural de Anastrepha ludens *(Loew) durante el período de oviposición. Promedios de 400 huevecillos.*

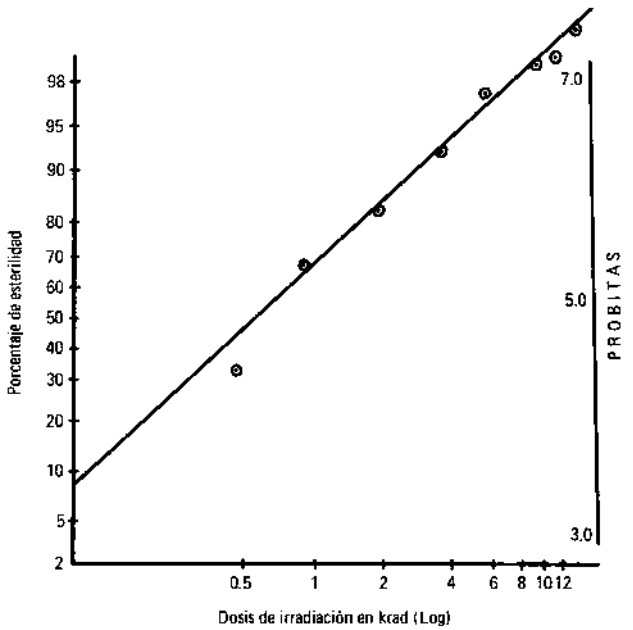

FIG.2. Esterilidad en machos de Anastrepha ludens *(Loew), a diferentes dosis de irradiación (proporción 1 : 1). Promedios de 400 huevecillos.*

la Fig.2. Los resultados promedio de tres pruebas indican que, de acuerdo con la tendencia de la recta, las dosis desde 2 krad ya ocasionan esterilidad alta y que el intervalo dentro del cual se puede elegir una dosis adecuada de irradiación para la obtención de machos estériles de *A. ludens* puede situarse entre 3 y 5 krad. Aparentemente, estos niveles de irradiación no modifican el comportamiento del macho, concuerdan en parte con los resultados obtenidos en forma preliminar por Enkerlin et al. [11] y parecen cumplir la condición de que la esterilidad debe ser obtenida sin modificar el comportamiento del insecto [12].

En un experimento hecho 20 días después de iniciada la oviposición se obtuvo una esterilidad muy alta (59 al 100%) en todas las dosis (0,5 a 12 krad), incluyendo al testigo.

En otras dos pruebas destinadas a determinar el efecto de la irradiación en las hembras provenientes de pupas irradiadas, se obtuvo que la hembra es mucho más susceptible que el macho, ya que las dosis de 0,5 a 1 krad afectaron significativamente a la fecundidad y a partir de 2 krad ya no hubo oviposición. El efecto de la irradiación sobre la fertilidad mostró la misma tendencia, pues desde el inicio de la oviposición se observó una esterilidad del 81% con 0,5 krad y del 90% con 1 krad, en contraste con el 13% en el testigo.

3.3. Competitividad del macho estéril

Los resultados de las pruebas hechas a 21–26°C y 65–70% de humedad relativa se presentan en las Figs 3 y 4.

Los resultados promedio de dos pruebas con la relación 9 : 9 × 1 : 1 aparecen en la Fig.3. La tendencia de la curva muestra que las dosis de 3 a 5 krad ocasionaron esterilidad elevada, superior al 90%, y que a medida que se incrementó la dosis, la esterilidad tendió a bajar (76% a 12 krad), por lo que se infiere un ligero aumento de cruzas entre individuos normales por falta de competitividad del macho estéril.

Los resultados de un experimento con la relación 19 : 19 × 1 : 1 se reflejan en la Fig.4, donde se observa que las dosis desde 2 krad en adelante ocasionan esterilidad muy elevada y que prácticamente no existe recuperación alguna de la fertilidad; sin embargo habrá que determinar el significado de los costos de obtención y manejo cuando se libera esta cantidad de material para determinar la conveniencia de su uso, o pensar en otras alternativas, tales como la irradiación de adultos o la irradiación de pupas y/o adultos en atmósfera de nitrógeno, neutrones de alta energía, CO_2, helio (He) o vacío parcial [13], para afectar en menor grado el vigor sexual, con lo que se logra la obtención de material más competitivo sin elevar significativamente los costos.

Al sospechar que las moscas utilizadas en estos experimentos no fueron de calidad óptima, recientemente (junio de 1981), después de hacer algunas

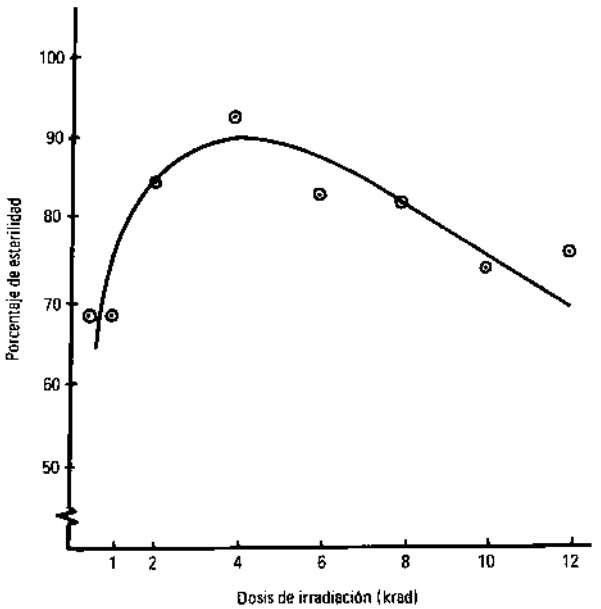

FIG.3. Esterilidad en Anastrepha ludens *(Loew), a diferentes dosis de irradiación (proporción 9:9 X 1:1). Promedios de 400 huevecillos.*

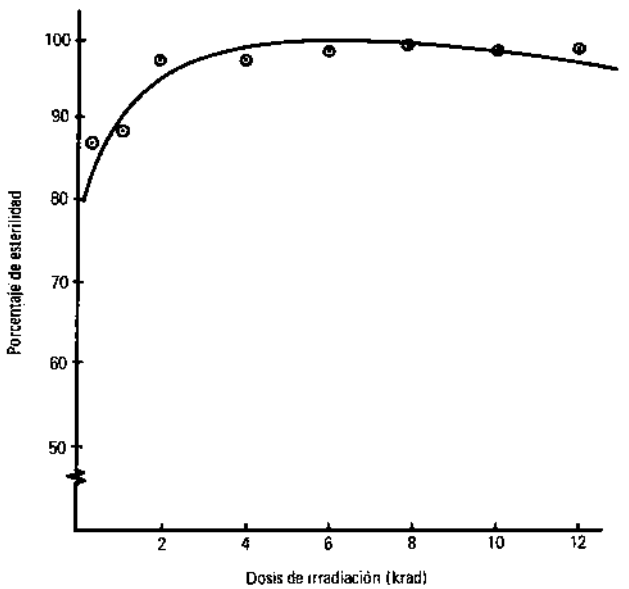

FIG.4. Esterilidad en Anastrepha ludens *(Loew), a diferentes dosis de irradiación (proporción 19:19 X 1:1). Promedios de 400 huevecillos.*

CUADRO I. INFLUENCIA DEL COLOR DE TRAMPA SOBRE EL NUMERO DE MOSCAS CAPTURADAS DE *Anastrepha ludens* DE DIFERENTE EDAD[a]

	Número total de moscas capturadas			
	Moscas de 2 a 9 días de edad		Moscas de 12 a 19 días de edad	
	Trampas amarillas	Trampas transp.	Trampas amarillas	Trampas transp.
	13	6	21	16
	21	8	30	9
	21	4	23	12
	10	4	37	7
	17	3	23	5
	24	17	29	6
	33	8	37	3
	36	9	14	3
	31	13	32	15
	35	7	51	11
	22	11	44	11
	37	6	32	7
	300	96	373	105
%	3,33	1,06	4,14	1,16
	MD = 17,0		MD = 22,3	
	ET_M = 2,48		ET_M = 6,8	

[a] Liberación: *moscas normales,* 9000 de 2 días y 9000 de 12 días de ambos sexos en cada caso. Temperatura: máx. prom. 30°C, mín. prom. 21°C. Humedad relativa: máx. prom. 85%, mín. prom. 63%.

modificaciones en la unidad de cría, se hizo una nueva serie de pruebas irradiando pupas en aire pero también en anoxia, siguiendo en general la metodología antes mencionada. Los resultados preliminares indican que las moscas macho fueron más competitivas, ya que la máxima esterilidad en las cruzas de 9:9 contra 1:1 se alcanzó con 8 krad, no habiéndose usado dosis más altas en estas pruebas. Las moscas cuyas pupas fueron irradiadas bajo anoxia sólo fueron ligeramente más resistentes.

CUADRO II. INFLUENCIA DEL COLOR DE TRAMPA SOBRE EL NUMERO DE MOSCAS CAPTURADAS DE *Anastrepha ludens* DE DIFERENTE EDAD[a]

	Número de moscas capturadas			
	Moscas de 2 a 9 días de edad		Moscas de 12 a 19 días de edad	
	Trampas amarillas	Trampas transp.	Trampas amarillas	Trampas transp.
	10	3	10	7
	10	4	12	8
	4	1	33	12
	5	2	4	3
	14	0	22	7
	21	7	35	17
	7	6	17	5
	15	3	18	15
	9	7	28	3
	18	7	16	11
	6	5	35	14
	9	2	4	9
	128	47	234	111
%	1,42	0,46	2,60	1,23
	MD = 6,67		MD = 10,25	
	ET_M = 3,08		ET_M = 6,19	

[a] Liberación: *moscas esterilizadas* con 6 krad, 9000 de 2 días y 9000 de 12 días de ambos sexos en cada caso. Temp. máx. prom. 34°C, temp. mín. prom. 23°C. Humedad relativa máx. prom. 83%, humedad relativa mín. prom. 45%.

3.4. Influencia del color de la trampa sobre la captura de adultos

Los resultados obtenidos al evaluar la influencia del color de la trampa sobre la cantidad de moscas capturadas, utilizando moscas normales, se presentan en el Cuadro I, e indican que el número de moscas de 2 a 9 y de 12 a 19 días de edad en las trampas amarillo solar fue significativamente superior al encontrado en las trampas transparentes de mica. Esto indica que el color amarillo por sí solo es un atrayente efectivo para adultos de *A. ludens*.

Los resultados obtenidos en otra prueba en que se utilizaron moscas estériles se presentan en el Cuadro II, en donde también se obtuvo que las trampas color amarillo solar capturaron poblaciones significativamente mayores que las trampas transparentes.

Los resultados obtenidos de las capturas con relación al sexo de las moscas que acudieron a las trampas de diferente color no indican diferencias significativas; en efecto, proporcionalmente se capturó igual cantidad de machos y de hembras por color y en las dos pruebas.

También es interesante reportar que siempre se capturó un número mayor de moscas de 12 a 19 días que de 3 a 11 días, asumiéndose que estas últimas, por ser más jóvenes, tuvieron mayor capacidad de dispersión.

Es importante también indicar que se capturó un número marcadamente superior al liberar moscas normales (874) que irradiadas (520), asumiéndose que la dosis de irradiación de 6 krad afecta negativamente a la visión de *A. ludens*.

3.5. Atracción sexual en el campo

Los resultados de la primera prueba se presentan en el Cuadro III, e indican que las trampas con atrayente (machos vivos) capturaron una población significativamente mayor que las trampas testigo, en una proporción aproximada de 5 : 1, o sea 1008 insectos en las trampas con atrayente contra 199 en las trampas testigo.

Por lo que concierne a insectos irradiados capturados en la misma prueba, los resultados muestran una tendencia similar, o sea que las trampas con atrayente capturaron significativamente más moscas que las trampas testigo, en una proporción de 3 : 1, es decir 362 insectos con atrayente contra 119 en el testigo.

Los resultados de la segunda prueba mostraron similitud con los de la prueba anterior, es decir que tanto para moscas normales como irradiadas las trampas con atrayente (machos vivos) capturaron poblaciones significativamente mayores que las trampas testigo.

Un aspecto importante encontrado en las dos pruebas fue que, al hacer un análisis del número total de insectos normales (1649) e irradiados (790), se observó en ambos experimentos un número de insectos normales significativamente mayor que el de irradiados; lo anterior indica que la dosis de 6 krad afectó de algún modo a los órganos de percepción al atrayente, o tal vez a la capacidad de vuelo.

Los resultados encontrados en la prueba con varios atrayentes al liberar y capturar machos normales de laboratorio se presentan en el Cuadro IV. Puede observarse que los atrayentes significativamente más efectivos e iguales entre sí fueron: machos vivos con 40, Torula con 34 y extracto de machos con 28 moscas capturadas respectivamente. En segundo término de efectividad quedó

CUADRO III. ATRACCION DE MACHOS VIVOS DE *Anastrepha ludens* (Loew) UTILIZADOS COMO CEBO AL LIBERAR 9000 MACHOS NORMALES Y 9000 MACHOS IRRADIADOS (MARCADOS) DE LABORATORIO[a]

	Número total de moscas capturadas			
	Normales		Irradiadas	
	Trampa con atrayente	Trampa testigo	Trampa con atrayente	Trampa testigo
	103	21	45	4
	96	39	26	17
	93	7	43	5
	87	15	30	10
	48	16	17	11
	85	16	30	10
	104	37	38	20
	107	17	37	16
	72	3	29	4
	74	10	15	9
	70	6	27	5
	69	12	25	8
	1008	199	362	119
%	11,20		4,02	1,32
	MD = 67,41		MD = 20,25	
	ET_M = 10,91		ET_M = 6,95	

[a] Temperatura: máx. prom. 36°C, mín. prom. 24°C. Humedad relativa: máx. prom. 83%, mín. prom. 45%.

el atrayente hembras vivas con 21 moscas y al final, con el número más bajo y sin diferencia significativa entre ellos, quedaron los tratamientos de extracto de hembras con 10 y el testigo con 5 moscas respectivamente.

Es importante el hecho de que el macho atrajo a machos en mayor número que otros atrayentes, indicando esto una alta probabilidad de la existencia de una feromona de agregación; los resultados concuerdan con los obtenidos por Leos [6].

CUADRO IV. EFECTIVIDAD DE DIFERENTES ATRAYENTES PARA LA CAPTURA DE MACHOS DE *Anastrepha ludens* (PROMEDIO DE CUATRO REPETICIONES) LIBERANDO 9000 MACHOS NORMALES DE LABORATORIO[a]

Atrayentes	Promedio de machos capturados por trampa[b]
Machos vivos	40,75 a
Torula (Prot. hidrol.)	34,00 ab
Extracto de machos	28,50 ab
Hembras vivas	21,75 bc
Extracto de hembras	10,25 cd
Testigo (trampa con pegamento)	5,75 d

[a] Temperatura: máx. prom. 33°C, mín. prom. 22°C. Humedad relativa: máx. prom. 87%, mín. prom. 59%.

[b] Los valores agrupados por la misma letra no son significativamente diferentes entre sí (probabilidad del 5%), según la prueba de Duncan.

Los resultados de la última prueba, en la que se liberaron y capturaron hembras normales de laboratorio, se reportan en el Cuadro V, e indican que la efectividad de los atrayentes fue similar a la de la prueba anterior. En primer término y con capturas significativamente mayores se encuentran los machos vivos y Torula con 24 y 18 moscas respectivamente. En segundo lugar quedaron el extracto de machos y las hembras vivas con 15 y 13 moscas respectivamente. Al final se encuentra el testigo con 5 moscas y el extracto de hembras con 3 moscas y sin diferencia significativa entre ambos.

En esta prueba también, el atrayente a base de machos vivos capturó la mayor cantidad de moscas, por lo que se puede asumir que en este caso obró como atrayente sexual puesto que las moscas liberadas y capturadas fueron hembras; estos resultados concuerdan con los obtenidos por Perdomo con *Anastrepha suspensa* (Loew) [14].

4. CONCLUSIONES

La esterilidad natural de *A. ludens* criada en laboratorio se incrementa rápidamente durante los primeros seis días y continúa alcanzando su máximo nivel alrededor del quinceavo día, pero tal vez su calidad no fue óptima.

CUADRO V. EFECTIVIDAD DE DIFERENTES ATRAYENTES PARA LA CAPTURA DE HEMBRAS DE *Anastrepha ludens* (Loew) (PROMEDIO DE CUATRO REPETICIONES) AL LIBERAR 9000 HEMBRAS NORMALES DE LABORATORIO[a]

Atrayentes	Promedio de hembras capturadas por trampa[b]
Machos vivos	24,45 a
Torula (Prot. hidrol.)	18,25 ab
Extracto de machos	15,50 b
Hembras vivas	13,00 b
Testigo (trampas con pegamento)	5,50 c
Extracto de hembras	3,75 c

[a] Temperatura: máx. prom. 31°C, mín. prom. 22°C. Humedad relativa: máx. prom. 87%, mín. prom. 52%.

[b] Los valores agrupados por la misma letra no son significativamente diferentes entre sí a una probabilidad del 5% según la prueba de Duncan.

El nivel de esterilidad del macho se incrementa rápidamente con el aumento de la dosis de irradiación, alcanzando más del 90% con 4 krad y alrededor del 100% con 6 krad en adelante.

Las dosis de irradiación entre 3 y 5 krad pueden ser suficientes para obtener alta esterilidad en los machos de *A. ludens* y afectará menos a diversos parámetros que la dosis de 6 krad usada actualmente en campañas de manejo o erradicación, pero esto depende de la calidad de la mosca.

No es factible una recuperación de la fertilidad del macho irradiado, aun con dosis muy bajas (0,5 krad), por lo menos hasta los 25 días de edad. La hembra es mucho más susceptible a la irradiación.

En pruebas de competitividad, la esterilidad se incrementó con el aumento de la dosis en la relación de 9 : 9 X 1 : 1, alcanzándose el nivel más alto a 4 krad; a dosis más altas se observó una recuperación ligera de la fertilidad, tal vez por una disminución de la competitividad del macho a altas dosis de irradiación.

En la relación de 19 : 19 X 1 : 1, la esterilidad se incrementó en forma más acentuada con el aumento de la dosis, pero los costos de una campaña se elevan al doble dada la cantidad de material estéril utilizada.

El color amarillo solar (COMEX 18–02) es un atrayente efectivo para adultos de *A. ludens* y captura alrededor de 3 a 5 veces más moscas que las trampas transparentes.

El color amarillo captura en la misma proporción ambos sexos de la mosca mexicana de la fruta.

El color amarillo capturó un número mayor de moscas normales que de irradiadas, indicando que la irradiación afecta a los órganos de visión de *A. ludens.*

En las pruebas de atracción sexual, el cebo a base de machos vivos atrajó el mayor número de mosca mexicana de ambos sexos; por lo que se asume que el macho emite dos tipos de feromonas, una sexual y otra de agregación.

En todos los casos las trampas capturaron un número significativamente más alto de moscas normales que de irradiadas, lo cual indica que la irradiación afecta en alguna forma a los órganos de percepción al atrayente.

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QUALITY CONTROL OF FRUIT FLIES IN A STERILE INSECT RELEASE PROGRAMME

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Abstract

QUALITY CONTROL OF FRUIT FLIES IN A STERILE INSECT RELEASE PROGRAMME.

The goal of a sterile fruit fly release programme is to eradicate, suppress or prevent spread of a wild population. This technique requires rearing, sterilizing and releasing large numbers of flies. Precautions must be taken that the rearing and handling procedures result in the efficient production of effective flies for this technique to be successful. The quantified assurance of production efficiency and of product (fly) effectiveness is termed quality control. Measures for production quality control include fecundity, egg hatch, rate of growth, larval production per unit of diet, pupal weight and adult eclosion. The primary attributes of fly vitality and behaviour (product quality) that are necessary for success in SIT (sterile insect technique) programmes are mobility, orientation, mating competitiveness and survival. Laboratory tests developed to measure these attributes include assays of irritability, flight ability, sustained flight rate, wing-beat frequency, visual acuity, pheromone response and mating propensity. These tests were modified and extended to determine their relevance to fly effectiveness in the field. Close correlations were demonstrated between results in laboratory tests and corresponding field performance. Thus, it seems possible to routinely monitor insect quality with simple laboratory tests complemented by less frequent verifications in field situations. Appropriate statistical procedures are available that identify cause:effect relationships, provide feed-back, and give predictive capability to the warning process.

General Concepts

Quality control in industry is a necessary part of the manufacture of products from heavy machinery to microprocessors. Most businesses today would be unable to compete without some sort of evaluation or feedback about their product.

In biological programs like the Sterile Insect Technique (SIT), the efficiency of the mass-rearing process is evaluated much like products on a production line. However, evaluations of the effectiveness of the product require unique criteria.

We are aware that research, at the start of a program, can never develop all of the information needed. If one waits until all of the questions are answered, programs will never get off the ground [1]. This was the case when the SIT was first conceived and tested. Certain information could only be gained by actually conducting the program. Other information was acquired by carefully planned field experiments and also by trial and error during the course of the program. As the technology increased with successive programs, the rate of success should have also increased. In most cases it did. However, some rather dramatic set-backs have occurred in recent years that have drawn attention to the most important and often neglected part of the programs, the vitality of the sterile insect being released.

To accomplish the release of sterile flies, large numbers (usually millions) of flies must be reared each week to overflood the wild populations. In the course of development of effective massrearing systems (i.e. efficiency, economy, convenience and factory environments) flies may sometimes be produced that are not effective in the field. To prevent, correct or minimize the chances of producting ineffective flies, the fact that such flies are being produced must be recognized. Many times, ineffective flies can be identified by visual examination. More often, however, the deficiencies are not so apparent and it becomes necessary to submit them to a series of tests to quantify their effectiveness. This quantification is termed product quality control.

The classic overflooding ratio of 9 sterile males to 1 fertile male as proposed by E. F. Knipling [2] has worked very well against the screwworm fly, Cochliomyia hominivorax (Coquerel). Even smaller ratios have had good effects against the tsetse fly. There is no precise information, however, on the proper overflooding ratio for fruit flies. For the melon fly, Dacus cucurbitae Coquillett, ratios ranging from 14:1 to 90:1 resulted in a successful eradication program on the island of Rota [3]. In later programs, ratios against the Oriental fruit fly, D. dorsalis Hendel, in unsuccessful compaigns, ranged from 27:1 to 64:1 on Saipan and from 39:1 to 322:1 on Tinian [4]. In Tunisia, a program against the Mediterranean fruit fly, Ceratitis capitata (Weid.), had ratios from 49:1 to 1 740:1 (average 430:1) that suppressed the population but did not eradicate it [5]. In Nicaragua, 90% control was achieved by an overflooding ratio of 1000:1 [6]. From these and other data, Hooper [7] has concluded that proper overflooding ratio for eradication of fruit flies is probably about 100:1.

We recognize that several factors may be responsible for the success or failure of any SIT program. Certainly, in our opinion,

fly effectiveness is a very important factor in view of the high overflooding ratios just described.

There are 2 types of evaluations appropriate for SIT programs: production quality control and product quality control. Production control provides regulation of the consistency of production output. It controls production by regulating and predicting the numbers of items produced and the timeliness of their production. The main aspects of production quality control include number of eggs produced per female, percentage egg hatch, larval growth rate, efficiency of the larval rearing system as determined by the yield (larvae produced per gram of diet), pupation rate, pupal size or weight, percent of adult eclosion, and adult longevity. These criteria are determined in most rearing facilities with generally accepted methodology. Control is achieved by manipulation of temperature, humidity, lighting conditions, dietary ingredients and handling techniques.

Product quality control (sometimes referred to as behavioral quality control) is an interaction of behavioral and physiological factors that determine the effectiveness of the product. The only purpose of mass-reared fruit flies used in this type of program is to carry a genetic load into wild target populations in intraspecific action. The released flies must have certain attributes to successfully complete their mission. These are adequate motility, orientation to the appropriate habitat (such as the host plants, the mating arena, and the oviposition substrates), adequate mating activity, mating compatibility with the wild target population and the ability to survive. The steps required for developing a product quality control program are 1) setting standards, usually based on components of the wild target population, 2) appraising conformance of the laboratory colony, 3) identifying detrimental factors and 4) determining corrective measures.

Motility

Good motility is important immediately after release. The fly must disperse from inhospitable environments to more hospitable sites where food and protection can be found and where flies can survive while their reproductive systems are maturing. Flies with a poor motility, for whatever reason, will probably not be able to locate host plants or the mating arena after release.

Several aspects of motility such as irritability, flight ability, flight rate, wing beat frequency and dispersal behavior can be measured in the laboratory and in the field.

Irritability. Irritability or the propensity to react to a stimulus is a desirable attribute for a fly. It increases the fly's ability to escape predators and to more quickly seek optimum environmental conditions. Docile individuals are more subject to mortality factors and thus would have reduced longevity.

The startle test operates on the sudden occurrence of a strong light which causes the flies to fly upward toward the light [8]. A startle index is achieved by the relative movement upward of the test group [9].

This test differs from the flight ability test in that it distinguishes those flies that can fly but will not (flight propensity).

Flight ability. The value of a simple test of flight ability was recognized during field experiments conducted in Guatemala in 1978 [9, 10].

The apparatus consists of a plastic petri dish (9 cm diam.) and a cardboard tube of 20 cm height which fits into the petri dish. The tube is painted vertically inside with FLUON® AD-1 that prevents flies from walking up the wall and escaping. Hence all flies that leave the system do so by flight.

The test is conducted by placing adults directly into the apparatus and determining the percentage of flies that escape by flight. A simple flight ability index is determined by dividing the number of flies escaping by the number of flies in the test X 100.

Flight rate. To determine the speed and duration of fruit fly flight, a flight mill must be employed. Flight mills consist of a rotor with a fixed arm attached to a low friction hub that rotates freely on a vertical axis when the insect is flying [11]. Mills may range from very simple models where rate is determined by an observer counting the revolutions against a stop watch, to elaborate models that interface with data processing devices. Data can be gathered on the effects of age, sex, diet, irradiation, etc., on speed and duration of flight.

Wing-beat frequency. The rate at which insects move their wings during flight is correlated with flight speed. 'Abnormal' wing-beat frequencies can often be used as diagnostic tools because they may indicate aberrations that are associated with abnormal physiological or morphological conditions [12].

Frequencies are determined either by use of a strobe light or more easily by analyses of the sound produced.

For testing, flies are glued individually to the head of an insect pin with rubber cement. When sound is to be recorded, the fly is mounted 2.5 cm above a condenser microphone with a flat frequency response. A paper held by the feet of the fly is removed and the fly begins to fly. One min. of flight sound is recorded on magnetic tape. Usually 10 flies are recorded for each test. The sound is analyzed for the following parameters: fundamental frequency, total power under the frequency curve, waveform distortion, and the frequency spread of the fundamental frequency per unit time per fly.

Dispersal. The real proof of whether a fly can and will fly in the field is to release it and see how far it can fly in a given

period of time. The only practical way to do this with large numbers of flies is to mark and release them and to recapture them at some distance at a later time. The flies can be released into suitable or unsuitable habitats but to rapidly ascertain only the dispersing ability, homogeneous unsuitable habitats may be more revealing. These flies are thus forced to disperse as they would if they were released in a nonhost environment.

Traps to recapture the flies may be arranged in concentric circles, grids or as a cross. Large numbers of flies released must be used so that the recaptures are sufficient for statistical analysis. Released flies must be identifiable by dye, paint or morphological markers when recaptured.

Factors affecting motility. A condition referred to as 'droopy-wing' in med flies has been described in Hawaii [13]. The seriousness of this condition ranges from normal appearing flies that can fly horizontally but which have little vertical lift to flies that cannot fly and cannot even hold their wings in normal position. A major cause of this condition seems to be agitation of pupae when they are separated from pupation media [14]. This causes separation of the attachment of the median dorsal flight muscles at a critical stage of development and is irreversible. This was also observed in Anastrepha suspensa (Loew) with pupal agitation and over-irradiation [15, Calkins and James, unpublished data].

Over crowding, irradiation and general rearing procedures also have negative effects on flight [9, 16, 17, 18, 19].

Habitat Interaction

Habitat interaction involves recognition of host trees, location of food sources and finally orientation to mating sites, i.e. trees that will most likely harbor virgin females. The attributes necessary for success that can be tested are visual acuity, spectral sensitivity and shape and color response.

Visual acuity. A system for measuring visual acuity and spectral sensitivity of insects is called the vision analyzer. It is used as a monitor of mass-reared insect colonies. Electroretinograms (ERG) measure the visual threshold of sensitivity and have been used in the past to compare the vision of laboratory-reared flies to their wild counterparts [20]. It may also be used to determine spectral sensitivity.

Flies, 2-3 days after emergence, are tested for visual acuity using an ERG technique. An electrode is placed into each eye of the insect and one eye is exposed to the test light. Neutral density filters are adjusted to get a response of 200 μV. The filter factor is recorded and averages and ranges are compared for each test population. The level of visual sensitivity for a population can be determined from a random selection of 5 insects [20].

Because both fruit and foliage of host plants are used as rendezvous sites for mating by many tephritids, color perception is very important. Therefore, sensitivity to an array of colors is determined in much the same way using color filters.

Shape and color response. Shape and color responses have been shown to be important measures of the ability of Dacus and Rhagoletis to orient to proper host trees and finally to fruit suitable for attraction to ovipositing females [21, 22]. Tests using models of the proper shape and color are coated with a sticky material and placed in a test area. Differentially marked flies from a test lot and from wild stock are released at some distance. Comparisons of the numbers of flies recaptured from each treatment would indicate the ability of released flies to orient properly.

Factors affecting habitat interaction. Diet, genetic inheritance, irradiation, larval density and handling methods can influence visual acuity [23, 24, 25]. Flies with unusual eye colors (frequently used as genetic marker strains) are lacking certain eye pigments and are thus more sensitive to light. The consequence is that under normal light conditions, they are partially blinded by the intense light and thus behave like flies with poor sensitivity. Laboratory flies also do not discriminate between colors or between curved or flat surfaces as well as wild flies [26].

Visual acuity is probably not as critical in massrearing facilities as in the field. As a consequence it is not selectively favored and the heritability of good vision may be reduced in the gene pool.

However, in the field where flies must react to visual cues in order to find host plants and mating arenas and to recognize potential mates and predators, poor vision is rapidly selected against. Thus, released flies with poor vision would probably not complete their intended function.

Reproduction

Probably the most critical period in the released fly's mission is concerned with mating. A series of behavior functions are necessary for successful insemination of fertile females by sterile males. The success or failure of a SIT program depends upon this.

Although fruit fly mating behavior is now being intensively studied, no one has really looked at the consequences of this behavior in terms of SIT. Most behavioral ecologists believe that the female controls the insemination event. This is because the investment in each unit progeny is smaller for males than for females. The male only donates sperm that is rapidly replenished. In species where a male has no 'family' obligation, his genes would have the best chance of being propagated if he mates with as many females as possible. On the other hand, the female has a large

investment; substantial amounts of her reserves are devoted to production and maturation of eggs. She has the added responsiblity of locating proper oviposition sites and of living long enough to oviposit. For her genes to survive, they must be paired with genes from a male which together impart to the progeny the 'tools' that give the best possible chance for their survival. Therefore in most cases, selection has favored genes for female discretion in selecting a mate.

With this background, let us look at a mating scheme that occurs in many species of mammals, birds and insects including Tephritidae. This is called 'lek behavior.' Lek is defined as 'a communal display area where males congregate for the sole purpose of attracting and courting females and to which females come for mating' [27]. This behavior has been recently found in several economic tephritid species including the medfly [28], the Queensland fruit fly [29], the apple maggot [30] and A. suspensa [31].

There are several consequences for lek mating behavior. The male must find the lek. Then he must compete with other males for the optimum calling station in the lek. The female that visits the lek for mating has the opportunity to choose a mate from an array of males. Therefore, he must appear fit in relation to other wild males. In a SIT program, if released sterile males are not nearly equal in courtship characteristics with wild fertile males in a lek situation, they will not be selected by the female as frequently as would be expected from the arithmetic ratio. In fact, if their courtship is significantly inferior, they may never participate in mating. This may help to explain the extremely high ratios of sterile to wild flies that are necessary for eradication.

Mating repertoire behavior of the medfly begins with males assembling in a lek. Intersexual aggression occurs when males vie for the optimum site and when territories are defended. Usually the larger males dominate. Males produce a pheromone to attract females. The chemical is dispersed into the airstream by wing fanning. A characteristic sound is produced simultaneously. Upon arrival of a female, the males face her and appear to direct the pheromone toward her with forward movements of the wings. A series of acoustic pulse trains are produced. The female approaches the chosen male then makes a series of lateral movements. The male normally moves to face her at all times. Finally the male will jump over her head and onto her back and copulate. Just prior to intromission, the wings appear to be used for balance but another unique sound is produced.

After a male arrives in the vicinity of the host plant, he must locate the best potential mating areas and orient to them. He must also be able to locate an active lek and to integrate into it. The possible ways are response to the male pheromone and/or to the acoustical pattern produced during calling. He then must compete for an advantageous site within the lek with wild fertile males.

Upon establishing himself in the lek, he should be able to dispense his pheromone at the proper time. This involves good pheromone production, proper pheromone dispersal mechanisms and proper periodicity of calling. When the female arrives, he must recognize her and begin his courtship. This requires propensities of good visual acuity and a proper courtship repertoire.

He must be compatible with the wild female so that he is readily accepted for copulation. Then he must successfully transfer live sperm and products of accessory glands that will cause a long mating refractory period in the female.

Some of the techniques that are used to measure the components are those also involved in other activities previously described (e.g. vision, orientation). The components directly involved in reproductive behavior are pheromone production and response, aggression, sound produced during calling, courtship, and precopula, speed of mating and time spent in copula.

The olfactometer is used in quality tests for quantitatively measuring the response of fruit flies to sex pheromones [32]. Flies moving toward the pheromone source enter the tube through a wire screening funnel and are retained. The pheromone source may be extracts of male flies, synthetic compounds or calling males.

The use of an olfactometer to quantify female response to male pheromone is a useful tool to elucidate changes in quantity and quality of pheromone produced by males in a mass-rearing system. The ideal system would use wild females tested against wild and production males. If wild flies are not available, the technique can be used to determine the effect of various stress factors on pheromone production or response, e.g. irradiation, changes in diet, larval crowding, etc.

To verify pheromone response in a more natural setting, more extensive tests are conducted in field cages or in the field [17]. To determine if males reared for release are as attractive to wild females as wild males, a choice test has been devised. It can be conducted in a field cage with known numbers of wild females or in host trees if the wild population is plentiful.

In host trees, the cages are paired, each pair in each tree serving as a replicate. Cages are checked at 15-min intervals and all wild females resting on the screened surface are collected.

When wild female populations are small, they may be restricted by using a large field cage. The cages containing males are arranged in a circle on the floor near the center of the field cage. The females are released into the cage. The females are collected at 15-min intervals.

This test can be used for simple comparisons of the responsiveness of several strains of females to several strains of males. It can also be used to determine the diurnal rhythms of male pheromone production and of female response.

Mating propensity. For a sterile released male to be competitive in an array of other males, he must have a high mating drive. If not, another male will copulate with the arriving female first. The mating propensity test measures the mating drive or speed of male flies [33, 34].

The experimental unit consists of a 30 x 30 x 40 cm plexiglass cage. Twenty-five unmated males and 25 virgin females, all reproductively mature, are placed into each cage. Mating pairs are removed at 10-min intervals. Each 10-min interval is given a certain weighted value, higher at the beginning and progressively lower later. This results in a Mean Mating Index (MI) that can range between 1 and 100. An MI of 100 indicates that all flies mated within 10 min of the test, whereas an MI of 1 indicates that no mating occurred for the entire test.

The relevancy of this mating test is shown when flies are placed in the large field cages and the test is conducted in the same manner as in the laboratory cages. In large arenas where crowding is not as important a factor, the test may reflect other components of behavior such as motility, lek formation and recognition, courting aggressiveness, acceptance and acceptability, and the rate at which these events occur. Detrimental effects are often magnified.

Duration of copulation. The length of time that males and females remain in copula is related to the successful transfer of sperm and readiness of females to remate. Relatively brief in copula often results in no or incomplete sperm transfer and very short refractory periods. Therefore, the length of time that flies spend in copula is a good measure of sexual compatibility.

Factors affecting reproduction. Because mating in fruit flies is a complex series of events, several individual factors have the potential to break the continuity and thus can disrupt or influence the mating success of sterile males.

Irradiation has been identified as a major cause of reduction in mating competitiveness [9, 35, 36, 37, 38]. Irradiation, as previously mentioned, reduces motility and vision. It certainly reduces longevity and probably vitality. It also changes acoustical signals used in courtship [39]. At any rate, it has a great influence on mating propensity as shown both in laboratory and field situations. Because increased dosage results in decreased competitiveness, the dose used is a trade-off between acceptable levels of sterility and competitiveness.

Diet affects courtship by changing acoustical signals. Vision is also important. If a male is approached by a female and is not able to track and respond to subtle signals because it is not able to see well, the female will reject him as a mate. Thus, flies with aberrations of vision are not as competitive [40].

The size of males plays a significant role in mate selection in A. supsensa and medfly. Small males have been observed to

readily mate with small females but were usually rejected by large females. On the other hand, large males readily mated with both large and small females. When large and small males were present together in a lek, large males were almost always more successful in mating. This may be due to larger males outcompeting smaller males for optimum sites or that females may discriminate on the basis of size [41, 42].

RAPID Method of Quality Control

A method of evaluating a profile of fly quality has been developed as a means for rapid evaluation without the need for sophisticated equipment and technology [9]. It allows the system to be implemented at any stage in the rearing, irradiation, release, and recapture regimes to locate problem areas in both time and space.

The system involves 5 laboratory tests which measures several key performance traits and characteristics comprising the quality profile. The components that were accepted and applied are:

Calibration of pupal size [9, 43].
Flight ability test.
Startle test for irritability.
Pheromone response test.
Mating propensity test.

These tests when summarised comprise the RAPID quality profile. The format is recommended for many of the major fruit fly laboratories. It contains the important information needed for direct comparisons with sets of data from other laboratories.

Process Control Charts

Another way of quantifying quality control is with the use of process control charts [44]. Process quality control charts are useful tools adopted widely in industrial production processes. They were among the first statistical tools to be introduced in the area of statistical quality. Process control charts are regular, orderly graphic representations and comparisons of quality characteristics. They can function in 2 ways: 1) they can be used to detect and identify assignable causes of variation, and 2) they can be used to warn of drift toward unacceptability due to nonrandom or assignable causes, and therefore can predict developing problems.

Process control charts represent 2 aspects of variability: (1) the differences between means of small samples from the grand mean of a large sample; and (2) the differences between ranges of small samples from an average range (Fig. 1). Thus, a chart of means displays the central tendencies of measurements of processes

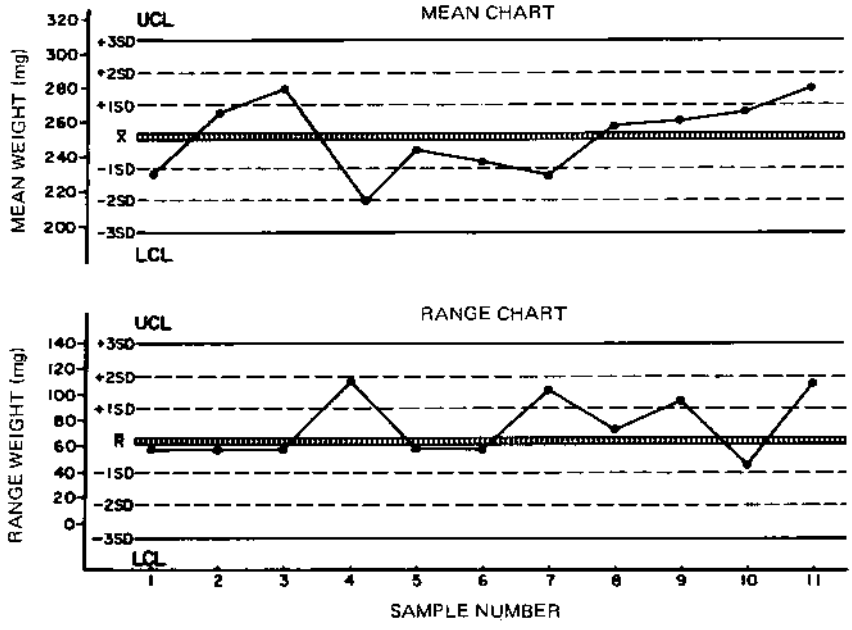

FIG.1. Typical process control charts with means and ranges plotted over time.

and a chart of ranges displays the spread of these measurements. The 2 charts are prepared using the large sample drawn initially for the frequency analysis. The grand mean and the average range are placed on the control charts as the expected central tendency and spread. The limits of normal variation established above and below the central line are typically plus and minus 3 standard deviations in industrial situations. Experience may suggest other limits for biological parameters. The control charts are used to plot sequential measurements of means and ranges made on small samples (usually 5 or 10) at regular intervals over a span of time. Thereby trends in the ability to control the process can be seen, and exceptional deviations can be spotted and related to production activities or mistakes. When mean or range values exceed the control limits, the process can be assumed to be 'out of control', representing a major problem on the production line. If variation is random and well contained within the control limits, control charting proceeds in order to maintain control. Even if the charted points fall within the control limits, care must be exercised to correctly interpret the occurrence of nonrandom drift toward one of the control limits.

Figure 1 shows mean and range charts for mating propensity of medfly. There were no alarming trends in either the mean or range charts. Deviations in both mean and range values were subsequently attributed to larval crowding. This closing of the 'feed-back loop' was stimulating to the rearing staff and resulted in renewed interest in the production process.

These charts thus not only serve as a method for closely evaluating the quality of the production and the product itself, but it gives needed feed-back to the rearing personnel. If the charts reveal no detrimental trends, the workers have a strong positive reinforcement and experience a feeling of accomplishment. If detrimental trends begin to appear, necessary steps can be taken to correct the causes. If the workers are then allowed to participate in rectifying the negative trends, it will demonstrate to them their importance in the program. The control charts, prominently displayed, will thus serve as score cards for each operation.

We suggest that process analysis should be applied to ongoing insect production systems. Reasonable quality of performance by the insects being produced is already assumed; however, one probably does not have quantifiable understanding of how production procedures result in reasonable quality, nor can one predict whether processes will continue to produce quality insects. Therein lies the first value of this analysis, its ability to identify causes of variability and its gift of prediction. The second value will be the quantification of reliable specifications for measurable traits that affect that quality (now only presumed or partly quantified).

Conclusions

Quality testing is a dynamic process. The tests described here should not be considered as the final complement. Tests will always be improved and new tests will be designed to quantify certain aspects unique to certain species.

However, as these tests are used routinely, an appreciation can be gained for the sometimes subtle effects of certain stresses which are present in a mass-rearing system but which have not previously been considered damaging to the flies' behavior. Thus the knowledge gained from a quality control program will give the insect rearing specialist more insight into the stresses which reduce the effectiveness of the final product.

NOTE : Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

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THE QUALITY PROBLEM IN OLIVE FLIES PRODUCED FOR SIT EXPERIMENTS

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Abstract

THE QUALITY PROBLEM IN OLIVE FLIES PRODUCED FOR SIT EXPERIMENTS.

Considerable research has been undertaken in the last two decades on the SIT method against the olive fruit fly. Nevertheless, whenever the method has been applied in the field, mixed results have been obtained. In a recent application on a small island, fruit infestation rose to high levels in October - November in spite of the fact that large numbers of sterilized flies were released from July to November for two consecutive years. Quality studies have revealed substantial differences between wild flies (W) and flies reared on an artificial system in the laboratory (L). Such changes were detected both at the genetic and the behavioural level. Among the behavioural changes detected, those of reduced flight ability, reduced visual sensitivity and different timing of sexual activity seem of importance to SIT. In small cages under laboratory conditions as well as in a large cage under natural-light conditions most matings between virgin sterilized L flies (LS) occurred two to three and a half hours before scotophase whereas most matings between virgin W flies occurred null to one and a half hours before scotophase. When flies remated, all matings occurred at the end of the photophase. In small laboratory cages, LS males proved very effective in overcoming the resistance of W females for rematings. This was not true in the large cage. It is very probable that the considerable mating-time separation observed between LS and W flies could be more intense under field conditions. Besides the above phenomenon, the data also suggested that the two fly-type females tended to mate with their own males.

In total, there are about 140 million cultivated olive trees in Greece which in 1980 produced about 305 thousand tons of olive oil and about 87 thousand tons of table olives, the total value being about 0.8 billion US dollars. Many millions of wild olive trees also exist, mostly in the mountains. Most of the cultivated trees are found in the southern half of Greece and the islands. In 1980, about 95 million olive trees were bait-sprayed some 2.7 times by organophosphate insecticides against the olive fly *Dacus oleae* (Gmelin) (Diptera: Tephritidae). Approximately 60 million olive trees were treated by spraying from the air. The total cost of the spraying operation (organized by the Ministry of Agriculture) was estimated at roughly 13 million US dollars in 1980.

In recent years, considerable effort has been devoted to developing control methods other than insecticides against this major olive fruit pest. In fact, the aim is to develop a pest management system which will protect the olive tree and fruit from their major pests, without, at the same time, producing major pollution problems in the olive tree agro-ecosystem. From the very beginning of this effort much interest concentrated on the use of SIT against the olive fly, a method which seems especially suitable for several of the Greek islands. At the beginning, most of research concentrated on artificial rearing and sterilization methods. Subsequently, considerable work has been devoted to comparisons between wild flies (W) and flies reared on an artificial system in the laboratory (L). Currently, research on artificial rearing continues to produce an effective mass-rearing system (reliable production of large numbers of healthy flies at low cost). Much research is also devoted to field applications and quality studies. At present, the rearing system in use [1] produces olive flies at a cost of about US $1 per 3500 pupae. Complete sterility of both sexes is achieved by 11 krad gamma rays in nitrogen one day before adult emergence [2].[1]

FIELD APPLICATIONS OF SIT

An attempt has been made to sterilize the natural population by a chemosterilant [3], and two two-year applications have been undertaken in which L flies were gamma sterilized (LS) and released. None of these applications produced unquestionable results to show that the method worked effectively in the field. The two release experiments were undertaken by the Demokritos Laboratory of Entomology in 1973–74 and 1979–80. In the first experiment the infestation of fruit in the release grove (about 600 trees located in Kassandra Halkidiki) was low in the first year but control groves did not show very high infestation either. In the second year the infestation reached very high levels in October in both release and control groves. The only difference observed was that the infestation rise in the release grove was delayed by about three to four weeks. In the first year, flies were irradiated as adults (1–5 days old) whereas in the second, flies were irradiated in the late pupal stage and kept thereafter in paperbags provided with adult food until all flies had emerged. Thus in both years a considerable number of flies must have mated before release [4; Zervas, unpublished data]. Approximately 150 000 sterilized adults were released every week from July to November in the first year, and about 250 000 pupae were placed in the release paperbags weekly from August to November in the second year [5].

[1] 1 rad = 1.00×10^{-2} Gy.

The second field application was undertaken during 1979–80 on a small island with about 400 olive trees near Eretria (Evia). In both years, late pupae were irradiated and transferred to field stations for emergence. In the first year, weekly releases started in June and finished in November, whereas in the second year they began in July. Approximately 12 million pupae were used in each year, from which about 50% overall adult emergence was recorded. In spite of the above massive releases the fly was not controlled in either year. In the first year the percentage of fertile infestation increased rapidly to about 60% in November. In the second year the infestation in November was 100%. On the contrary, the infestation in the mainland across the island was kept below 10% by four aerial bait-sprays in the first year, whereas in the second year it rose quickly to about 75% in November due to poor timing and non-uniformity of the last spraying in October (Economopoulos, Haniotakis, Manoukas, Mazomenos, Tsiropoulos, Tsitsipis, Tsokos, and Zervas, unpublished data).

QUALITY STUDIES

The most important data obtained so far are presented below.

Bacterial infestation

During the second field application (1979–80) serious bacterial infestation of pupae occurred in the second half of September and in November of 1979 (field emergence 16 to 36%), and at the end of July and in August of 1980 (field emergence 22–43%). The bacterial problem was identified in 1980 as *Pseudomonas aeruginosa* which turned pathogenic due to stress conditions in the rearing system (G. Thomas, personal communication).

Genetic structure

Electrophoretic studies on gene frequencies of certain enzymes showed extensive differences between L and W flies. When W flies from different localities (even very distant ones) were compared, the difference was not as big as that between L and W flies. Such changes were found to occur very fast following colonization (2–3 generations only) [6,7; Zouros, Loukas, Mazomenos, and Economopoulos, unpublished data].

Thus, it appears that substantial changes in gene frequencies occur due to strong selection powers in the fly colony. The most serious obstacles encountered when W flies are introduced in the rearing system are oviposition on paraffin or ceresin domes or cones, and larval growing in artificial diet.

Flight capacity, dispersal

When L and W flies were compared in a flight mill, L flies travelled much shorter distances than W flies. On the average, L males and females covered about 2250 and 4200 m respectively in 24 h, whereas W males and females covered about 6800 and 10 000 m respectively [8]. In field studies it was found that when both L and W flies were released simultaneously at the same point, both flies were recovered in about equal numbers in short distances. In long distances, the majority of recovered flies were W flies [9, 10]. Thus, it appears that in long range dispersal L flies are inferior to W flies.

Visual sensitivity, host detection

Field experiments showed that the response of L flies to certain colours was considerably different from that of W flies. The responsiveness to red showed the greatest difference between L and W flies. The red colour was found to be neutral for W flies as was also grey and clear Plexiglass. L-type flies showed considerable responsiveness to red although they did not respond to grey and Plexiglass. When the eyes of the two fly types were examined, the W-fly eyes became irridescent blue-green within a day after emergence, whereas the L-fly eyes remained dull red throughout life. When W flies were reared on artificial diet for one generation their eyes became reddish, whereas they remained irridescent blue-green when the flies were kept on olives [11].

In other studies, the spectral sensitivity in three age groups of L and W flies was studied by the electroretinogram technique. W-type flies and L flies reared in the laboratory for about 20 and 130 generations were compared at 1, 10 and 22 days of adult life. A tendency was found for older flies to exhibit higher spectral sensitivity. L-type flies exhibited lower spectral sensitivity than W flies of similar age. This reduction in visual sensitivity was more pronounced in L flies kept on artificial rearing for many generations. No major differences in the spectral sensitivity pattern in all three strains were observed. All flies showed peak sensitivity at 490 nm (Remund, Economopoulos, Boller, Agee, and Davis, unpublished data).

In host detection studies it was found that both L and W flies were equally able in distinguishing a real olive from a wooden model after arrival. However, both sexes of L flies showed comparatively higher attraction to red models than W flies. It was concluded that females located the olive fruit by visual characters, in particular shape, colour and size [12]. In another study it was found that sexually mature L and W flies responded similarly to olive tree foliage, and both distinguished equally well real foliage, probably by its colour, from painted surfaces or empty space. No response of either fly type to olive fruit odour, and no apparent effect of odour from leaves and twigs on either sex were detected [13].

Mating activity in mixed populations of LS and W flies

The poor results of the 1979–80 SIT experiment, in spite of very large numbers of LS flies released over a rather small area, initiated a series of experiments in small laboratory and large field cages in which we studied mating activity by direct observation in mixed populations of LS and W flies. In small-cage studies with separately maintained L and W flies, it had already been found that L flies start their mating activity considerably earlier than W flies (Zervas, unpublished data). Although the results of Experiment A are in press as part of Ref. [14], they are included here for comparison with Experiments B and C.

Materials and methods

LS-type flies used had been reared for several generations in the laboratory [1] and were sterilized by ^{60}Co gamma rays [2].

W-type flies were collected as larvae ready to pupate from olive fruits harvested in olive groves about 40–60 km northeast of Athens in the autumn or the beginning of winter. In the laboratory experiment (A) we used 12L/12D, 25°C, and 60% relative humidity environmental conditions. Fifty 8-9-day-old LS flies were mixed with 50 15-20-day-old Wflies in each of four 42 X 26 X 26 cm Plexiglass cages (L flies mature sexually earlier than W flies [4; Zervas, unpublished data]). Flies were mixed at a 1 : 1 sex ratio. For Experiments B and C we installed a plastic-screen cage, with dimensions roughly 2 X 2 X 2.5 m, in a greenhouse to avoid low temperatures (experiments run in autumn and winter). Four small potted olive trees were placed in the cage. In Experiment B, 800 roughly 5-day-old LS flies were mixed with 160 roughly 20-day-old W flies at a 1 : 1 sex ratio. In Experiment C, 160 roughly 18-day-old W flies at a 1 : 1 sex ratio were mixed with 400 roughly 9-day-old LS males. The greenhouse temperature fluctuated between 15°–25°C and relative humidity between 60–80% during Experiment B (windows open, no air-conditioning provided). The temperature and relative humidity were at about 18°–21°C and 40–50% respectively during Experiment C (windows closed, heater operating). Experiment B began at the end of November 1980, and Experiment C at the end of January 1981. Light intensity was about 1200 lx in Experiment A. In the large cage in the greenhouse, light intensity fluctuated depending on the day (clear, cloudy, rainy, etc.). On a clear day in the autumn it ranged between 1000–7000 lx about 2 h before dark, and 20–30 lx just before dark (difficult to detect matings) in the four corners of the ceiling of the cage. Flies in the greenhouse cage were practically always found on the ceiling in the areas of high light intensity (E, S, SW) and almost never on the potted olive trees. In all experiments W flies had been marked with a small spot of Mo-Lak S.p.A. yellow enamel on the notum [15]. Matings in all experiments were recorded daily in the last five hours of photophase at 15-min intervals, and they were

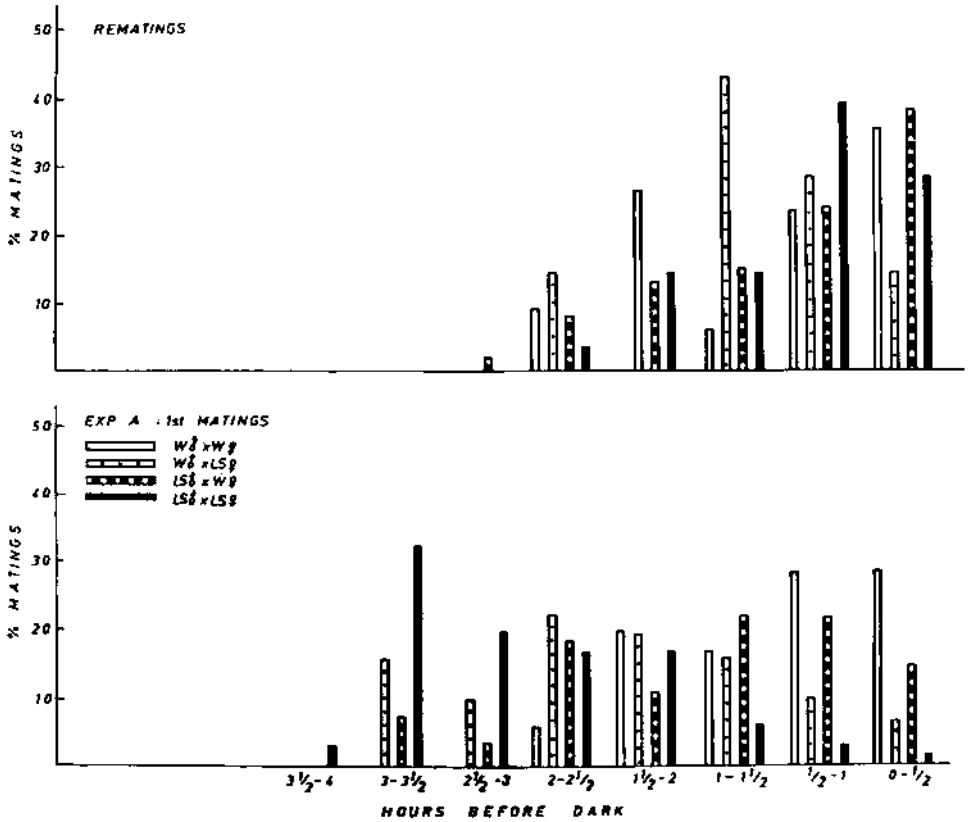

FIG.1. Percentage of matings at various times before scotophase in the different mating combinations of Experiment A (small cages, laboratory L/D regime). Sexually mature virgin flies mixed together at 1 : 1 : 1 : 1 ratio (LS♂/W♂/LS♀/W♀; LS = laboratory-reared gamma sterilized, W = wild flies).

subsequently isolated for the rest of the day in glass vials (mating duration had been found well above one hour [16]). Dead flies were recorded daily. Cages were provided with water and protein diet. All experiments lasted 12 days.

Results and discussion

The distribution of matings in each of the four (Experiments A, B) or two (Experiment C) possible mating combinations is shown in Figs 1–3. In the laboratory experiment (A), an extensive time-separation of matings in the two fly types is obvious during matings between virgins (WW versus LSLS matings).

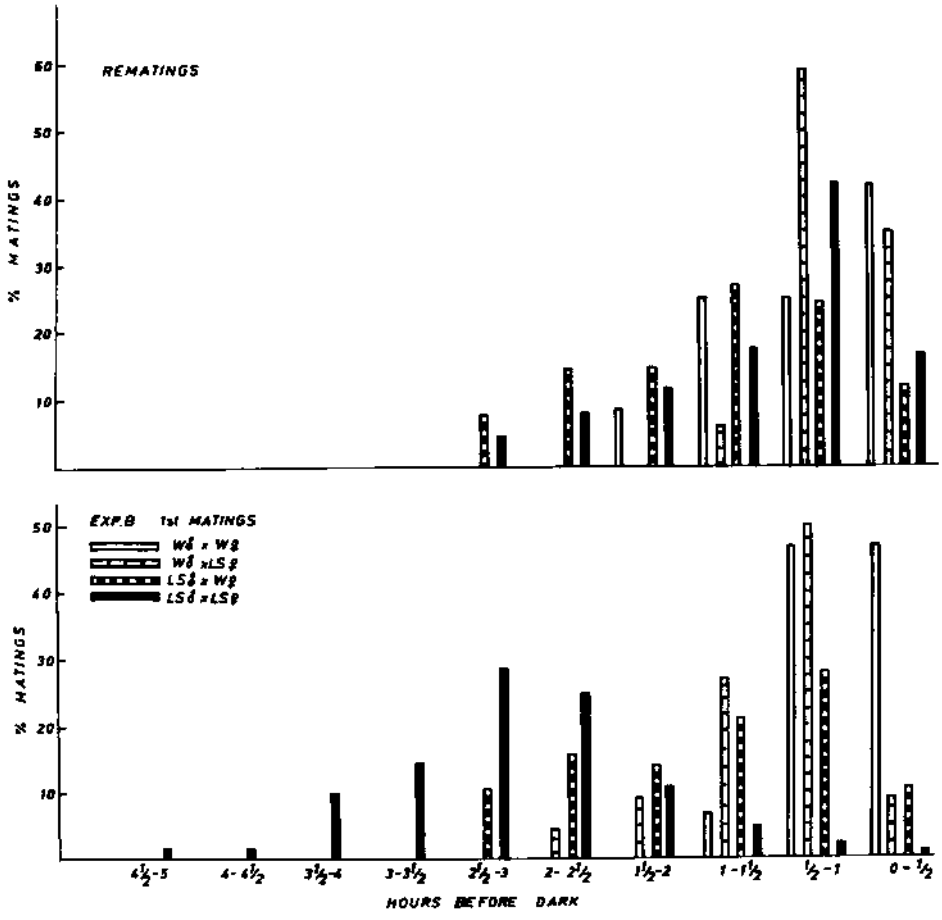

FIG.2. Percentage of matings at various times before scotophase in the different mating combinations of Experiment B (large cage, natural L/D regime). Sexually mature virgin flies mixed together at 5:1:5:1 ratio (LS♂/W♂/LS♀/W♀; LS = laboratory-reared gamma sterilized, W = wild flies).

LSLS-type matings started three and a half to four hours before scotophase with about 55% of them occurring two and a half to three and a half hours before dark. On the other hand, about 55% of WW-type matings were recorded null to one hour before dark. Cross matings gave intermediate results with LS♂ W♀ approaching the WW pattern and W♂ LS♀ approaching the pattern of LSLS matings. This indicates that the female's role was probably more important in determining if and when a mating would have occurred. At remating, all matings concentrated at the end of the photophase (Fig.1). In Experiment B, the separation between LSLS- and WW-type matings was more pronounced than in Experiment A.

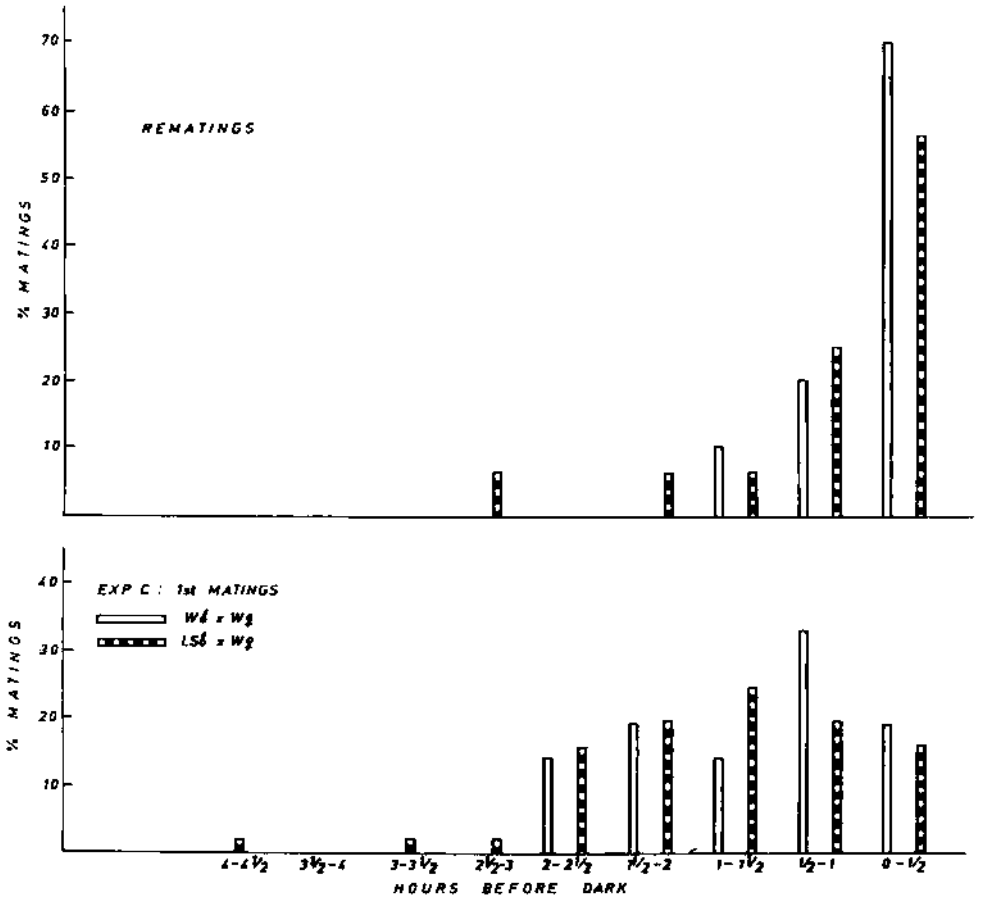

FIG.3. Percentage of matings at various times before scotophase in the different mating combinations of Experiment C (large cage, natural L/D regime). Sexually mature virgin flies mixed together at 5:1:0:1 ratio (LSδ/Wδ/LSφ/Wφ; LS = laboratory-reared gamma sterilized, W = wild flies).

LSLS-type matings started four and a half to five hours before dark with about 55% of them occurring two and a half to five hours before dark. Almost 90% of WW matings occurred null to one hour before dark. In Experiment B, both cross-matings approached the WW mating pattern. At remating, all matings concentrated again at the end of the photophase (Fig.2). In Experiment C, matings of W females with either LS or W males were similarly distributed during the last two and a half hours of photophase. Upon remating, both mating types concentrated in the last hour of the photophase (Fig.3).

TABLE I. PERCENTAGE OF TOTAL MATINGS RECORDED IN EACH MATING COMBINATION IN MIXED POPULATIONS OF LS AND W FLIES UNDER LABORATORY AND NATURAL L/D REGIME (12L/12D AND WINTER, RESPECTIVELY)

Experiment[a]	Insect matings[b]	Insect-type ratio[c]	Percentage of total matings			
			W♀ X		LS♀ X	
		LS♂ /W♂/LS♀/W♀	LS♂	W♂	LS♂	W♂
A	1st	1.0 /1.0/1.0/1.0	16.9	23.1	40.6	19.4
	total	1.0 /1.0/1.0/1.1	48.3	18.2	23.9	9.7
B	1st	5.0 /1.0/5.0/1.0	12.9	3.4	78.8	5.0
	total	5.8 /1.0/6.0/1.1	15.1	4.2	74.8	6.0
C	1st	5.0 /1.0/0.0/1.0	73.1	26.9	–	–
	total	4.2 /1.0/0.0/0.9	70.2	29.8	–	–

[a] A = in the laboratory, small cages; B and C = in the greenhouse, large cage.
[b] 1st = between virgins; total = 1st matings plus rematings.
[c] Ratio of survival days.

When the percentage of each mating combination is calculated in Experiment A (expected value 25%), LSLS matings were found to be the most numerous whereas LS♂ W♀ were the least numerous in the matings between virgins. In the overall experiment, LS♂ W♀ matings gave the highest record and W♂ LS♀ the lowest. It is concluded that in the small cages in the laboratory, LSLS matings dominated when insects were virgin, mainly because of the earlier sexual maturation of LS flies within the day. At remating, when sexual activity concentrated at the end of the photophase, LS♂ W♀ matings dominated probably because of the LS male's effectiveness in overcoming the W female's resistance to repeated mating under the laboratory conditions that were unfamiliar to W flies. The opposite was true in W♂ LS♀ rematings. It should be noted that in the olive fly the male had been found able to mate every day [17] whereas the female had been found unable to mate very frequently [15, 18–20]. In the greenhouse Experiment B, no increased effectiveness of LS males was observed. More probably, W females were better able to avoid repeated matings than in the small laboratory cage. Although the percentages of total female matings were much as expected according to female-type ratios (83 and 17% for LS and W females

TABLE II. FEMALE MATING FREQUENCY AND MATING RATIO FACTOR IN MIXED POPULATIONS OF LS AND W FLIES UNDER LABORATORY AND NATURAL L/D REGIME (12L/12D AND WINTER, RESPECTIVELY)

Experiment[a]	Insect matings[b]	Days between matings		Mating-ratio factor (LS♂/W♂ matings/LS♂/W♂ days[c])	
		W♀	LS♀	W♀	LS♀
A	1st	–	–	0.73	2.09
	total	3.6	6.7	2.65	2.46
B	1st	–	–	0.76	3.15
	total	5.9	7.1	0.62	2.15
C	1st	–	–	0.54	–
	total	7.1	–	0.56	–

[a] A = in the laboratory, small cages; B and C = in the greenhouse, large cage. For insect-type ratio in the different experiments see Table I.

[b] 1st = between virgins; total = 1st matings plus rematings.

[c] The ratio of matings over the ratio of survival days should equal 1 when insects do not differ in their sexual behaviour performance. For mating and survival ratios see Table I.

respectively), LSLS matings were at much higher percentages than expected (compared with LS♀ W♂ matings), and similarly WW matings were at higher percentages than expected (compared with W♀LS♂ matings). This was again probably caused by the different timing of mating in the two fly types. In Experiment C, W females mated again with their own males at higher than expected percentages (Table I).

The intermating period of females was similar in both female types in all experiments except W females of Experiment A. As discussed above, W females were probably forced to mate frequently in the unfamiliar environment of small cages by the LS males highly effective in such an environment. When the mating-ratio factor in the two female types is compared, we conclude that, except for rematings of Experiment A, LS males were not very effective in mating with W females, whereas they were very effective in mating with their own females. This was apparently, in part, the result of mating-time separation in the two flies. The fact that the situation did not improve in Experiment C where no LS females were present suggests that other reasons, apart from mating-time synchronization, also contributed to the reduced effectiveness of LS males in the large cage (e.g. reduced vigour due to artificial rearing and/or irradiation, altered sexual behaviour, etc.) (Table II).

In conclusion, it appears that the mating activity between LS and W flies in mixed populations changed considerably from small laboratory cages to the large natural-light cage. The different mating time observed in matings between virgins of the two fly types in the mixed populations could be very important in SIT applications, since in the field flies will probably be widely separated and females will probably mate only once in their lifetime [19]. Considering that even in our large greenhouse cage most of the flies were very near to each other (because they concentrated on the cage ceiling) which facilitated perception and mating attempts, we realize that in the olive tree environment frequent encounters and cross matings, especially of the LS♂ × W♀ type, may be too few to have a substantial impact on the natural population growth.

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INVESTIGATIONS ON SOUND PRODUCTION OF *Ceratitis capitata* L.

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Abstract

INVESTIGATIONS ON SOUND PRODUCTION OF *Ceratitis capitata* L.

In the framework of an IAEA service contract (TC 2235/TC) several experiments were made to determine if changes in sound production could be indicative of changes of quality in various strains of the Mediterranean fruit fly, *Ceratitis capitata*. The studies have been carried out in close co-operation with the USDA, Insect Attractants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida (D.L. Chambers, J.C. Webb, C.O. Calkins). The investigations show clearly that at present the bioacoustic method can only be used in connection with other methods of quality control. Nevertheless it will be necessary to continue such studies, especially with respect to the progress of genetic engineering and the use of different morphological strains of *Ceratitis capitata* in connection with the use of SIT.

1. INTRODUCTION

In the framework of an IAEA service contract, several experiments have been made to determine if changes in sound production could be indicative of changes of quality in various strains of the Mediterranean fruit fly (medfly), *Ceratitis capitata*.

The studies have been carried out in close co-operation with the United States Department of Agriculture, Insect Attractants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida (D.L. Chambers, Director), particularly with J.C. Webb.

Various investigations have been conducted during the last year on possibilities of using the bioacoustic method for quality control [1], particularly on *Ceratitis capitata* L., in the bioacoustic laboratory of the Bundesanstalt für Pflanzenschutz, Vienna. Some of the results are summarized.

2. METHODS AND MATERIAL

Recording of the various sounds was carried out using the following techniques:

Microphone: Sennheiser MKH 105
Tapes: Ampex 641
Tape recorder: Stellavox Sp 7
Tape speed: 3.75 ips
Input sensitivity: + 10 – + 15 db

All records were made using the same technique, so that comparable results were obtained by a method which could be easily repeated. Climatic conditions during the experiments were: room temperature, 22°–28°C; relative air humidity, 50 – 60%; illumination: mercury light or a 200 W variable bulb.

2.1. Material for the experiments

Males and females from the Seibersdorf laboratory and other origins were used. The flies were segregated according to sex two days after eclosion and brought into the recording room for acclimatization on day 6 – 7. For the purpose of sound recording 3 – 5 males and 2 females were placed in a special recording box (8 × 8 × 8 cm).

The distance between the flies and the microphone was 4 to 6.8 cm.

2.2. Recording process

After positioning the flies in the sound-proof recording room, a light was switched on immediately before recording. The beginning of sound production was detected by means of a control loudspeaker, headphone, TV monitor and oscilloscope in the recording room.

Each experiment was repeated four times and each experiment consisted of a minimum of 25 recordings of different flies.

3. RESULTS

The following is a description of various sounds and behaviour.

3.1. Calling sound

This sound is made coincident with the excretion of a sex pheromone from the male [2]. The pheromone gland is extruded from the tip of the abdomen

and the fly begins to fan its wings. This may be a means of dispersing the pheromone within the canopy of the tree.

This behaviour has been termed 'calling behaviour' in other insects which only possess pheromone. Because this sound is so closely associated with the pheromone dispersion (calling) we have referred to it as the calling sound [1].

3.2. Courtship sound

This sound is usually made by the male after the female responds to the calling sound. As the female approaches the calling male, the male turns to face her and at the same time appears to direct or push the pheromone towards her from between his legs in a series of buzzes made by the wings. As the female moves, the male continues to position himself directly in front of her and appears to dance, at the same time continuing to emit the characteristic 'buzzes'.

We have termed this the courtship sound because the male is courting the female at this point.

A very similar sound and behaviour of the female has been observed only in the Seibersdorf strain and by Rolli [3]. The significance of this is not yet known.

3.3. Aggression sound

This sound, made by the female, is associated with head-butting by the female in rejecting a zealously courting male. It is produced as a very short burst of sound made by the wings.

3.4. Sound associated with copulation

This sound is made by the wings of the male during the mounting of the female. It may only be an incidental sound made in an attempt to stabilize himself on an unstable, moving female.

4. PROCESSING THE ANALYSIS

The tapes with records and all protocols were sent to J.S. Webb at the Gainesville Laboratory for analysis via the IAEA. The analysis was carried out by the following techniques:

(a) Sonograms (University of Florida, Gainesville, T.J. Walker)
(b) Luminoscripts (USDA Laboratory, Gainesville, J.S. Webb)
(c) Spectral analysis (USDA Laboratory, Gainesville, J.S. Webb)

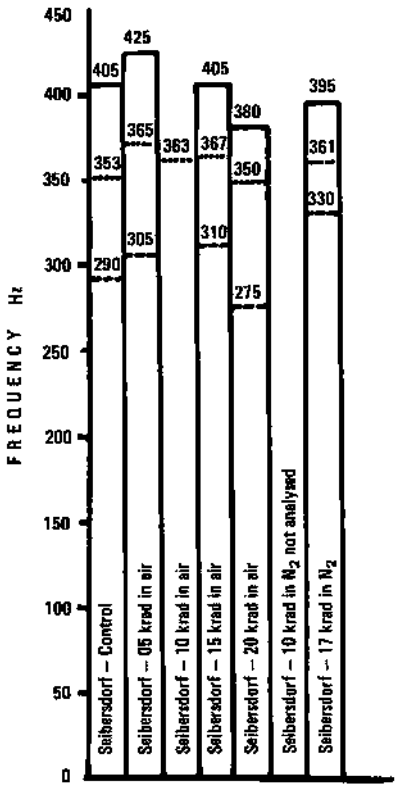

FIG. 1. The effect of increasing radiation doses on the minimum, mean and maximum frequencies of calling sounds of the Mediterranean fruit fly.

The spectral analysis (pulse train) was recorded by a computer, protocolled and stored.

The interpretation of sound production was carried out taking the following into consideration:

(A) Frequency distribution of sound frequency
(B) Frequency analysis
(C) Pulse train duration
(D) Pulse train interval
(E) Number of pulse trains
(F) Number of pulse/pulse trains
(G) Averaging area under curve

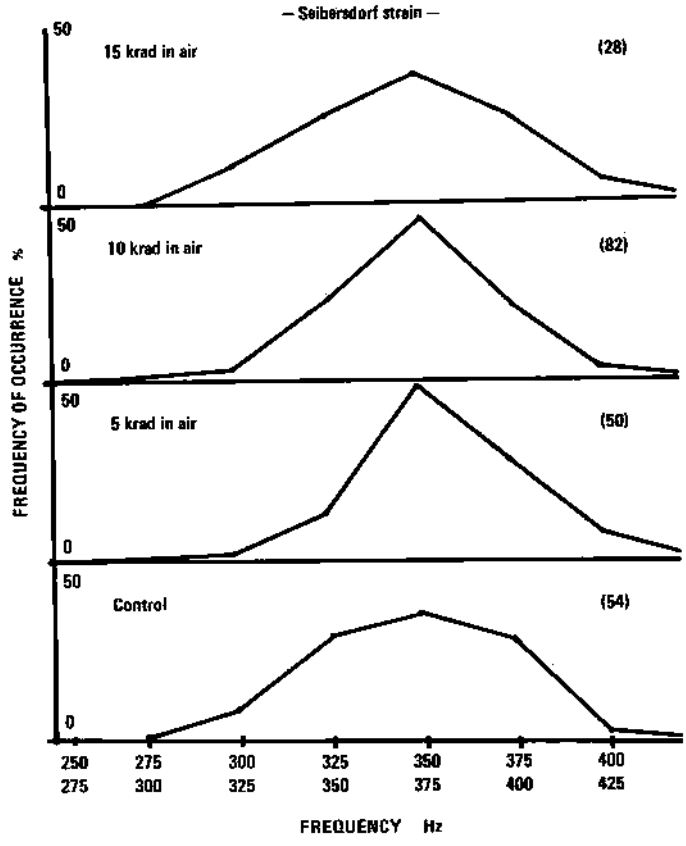

FIG.2. Distribution of the frequencies of calling sounds of the male Ceratitis capitata *Wied. at different irradiation doses.*

In the target paper only the following items were taken into consideration:

(a) Frequencies of calling sound
(b) Frequencies of courtship sounds

5. RESULTS

5.1. The effect of increasing irradiation doses on the frequencies of calling sound

There were no detectable changes in calling sounds in the check-population (Seibersdorf strain) from the onset of sexual maturity to the adult age of 17 days.

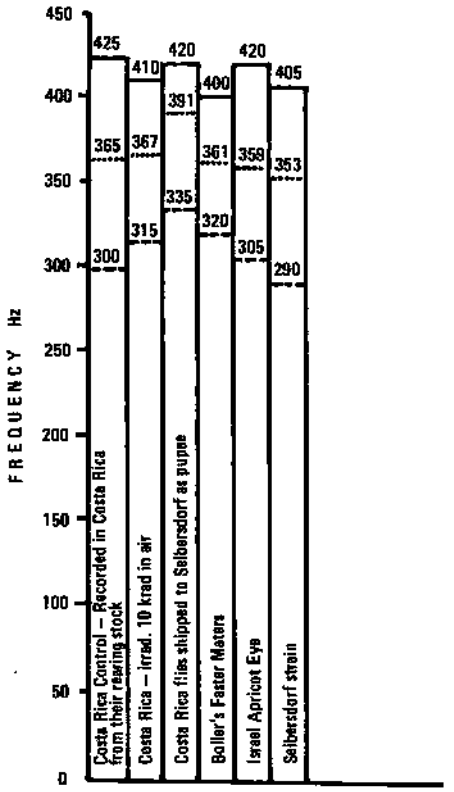

FIG.3. Minimum, mean and maximum frequencies of the calling sounds of the Mediterranean fruit fly from different rearing regimes.

However, there were significant differences after treatment with increasing irradiation doses when the *means* were taken into consideration (Fig.1).

Although the results of the analyses of the minimum and maximum frequency values have not been completed, it appears that a dose of 20 krad in air has some effect and may be approaching the level where disruption of the fundamental frequency occurs.[1]

Analysing the distribution of the frequencies of calling sound it appears that there is a spreading out of the distribution curve with increasing doses; however, the non-irradiated control is similar to the highest dosage (Fig.2).

[1] 1 rad = 1.00×10^{-2} Gy.

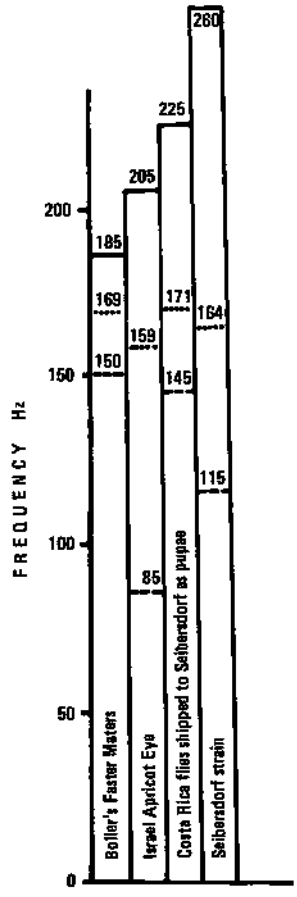

FIG.4. Minimum, mean and maximum frequencies of the courtship sounds of the Mediterranean fruit fly from different rearing regimes.

5.2. The effect of increasing irradiation doses on the frequencies of courtship sounds

There was no significant effect from different doses on the fundamental frequency of the courtship sound. There appear to be no changes in frequency due to irradiation damage.

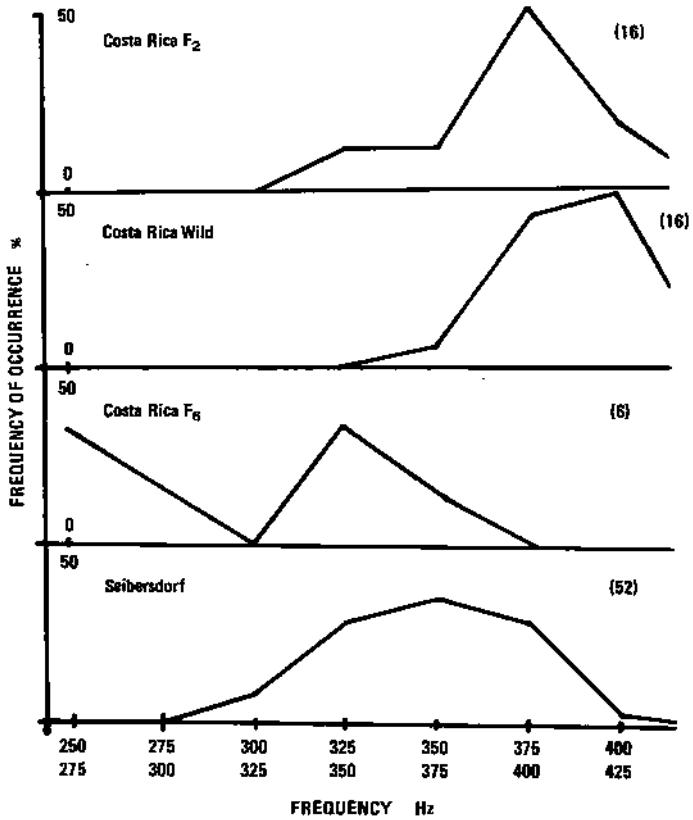

FIG.5. Distribution of the frequencies of calling sounds of the male Ceratitis capitata *Wied. from different geographical areas.*

5.3. Investigations on irradiated and unirradiated flies from various origins (strains)

The aim of these investigations was to study the differences in calling or courtship sounds using flies from various strains. The following strains were studied:

(1) Costa Rica factory strain (irradiated or unirradiated)
(1a) Costa Rica wild flies strain
(2) Seibersdorf strain
(3) Swiss 'fast mating strain'
(4) Israel apricot eye strain

J.S. Webb recorded mass-reared males unirradiated in the Costa Rica medfly factory. These data were used to compare with the Costa Rica wild flies strain, which was shipped to Seibersdorf and recorded in our bioacoustic laboratory.

Later a comparison was made between the Swiss 'fast mating strain' (Boller), and the Israel apricot eye strain (Rössler) [4].

The results of these investigations are shown in Figs 3–5.

There were no statistical differences between any of the strains in fundamental frequencies of the calling or the courtship sounds. The Costa Rica wild flies, however, had a somewhat higher calling frequency than the rest.

The distribution of frequencies in the calling sound among the flies from different strains shows that the Costa Rica laboratory and Costa Rica wild strains have a very similar pattern. The Costa Rica F_6-strain has a very strange frequency pattern which cannot be explained.

The Seibersdorf production strain is not as clearly defined as the rest, and the Swiss 'fast mating strain' is very similar to the Seibersdorf strain.

6. SUPPLEMENTARY OBSERVATIONS

6.1. Atypical behaviour of females, especially those originating from mass breeding

A typically 'male behaviour' of females has been observed in the case of individuals reared under laboratory conditions. These females imitate a calling sound as well as a copulation sound and both can be differentiated from the typical male sound in the following way:

(a) Calling sound varies in pitch
(b) Courtship sound is irregular and abnormal in comparison with the male sound

Behaviour similar to that of males also occurs. These atypical behaviour patterns are released by the presence of courting males or atypical females of the same age.

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FACTORS INFLUENCING STERILITY AND VITALITY OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* Wiedemann

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Abstract

FACTORS INFLUENCING STERILITY AND VITALITY OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* Wiedemann.

Males of *Ceratitis capitata* Wied. previously reared on three different larval media (molasses, sugar and glucose) were irradiated as old pupae with 7, 9 and 11 krad of gamma radiation. Results indicated that egg hatchability and male survival did not differ significantly in the three media at the three applied doses. Mating competitiveness of 9 krad-irradiated males at the ratio 3:1:1 (irradiated males : normal males : normal females) did not differ when using the different larval diets. When pupae were irradiated with the three doses, each given at three different dose rates, i.e. 7, 30 and 97 rad/s, a gradual decrease in egg hatchability was observed as the dose rate was increased. Adult survival was not affected by differences in either dose rate or in the radiation dose. Mating competitiveness of 9 krad-irradiated males was not affected by changes in dose rate. Old pupae were irradiated with 9 krad in three fractions at one-day interval between each treatment. Egg hatchability, male survival and male competitiveness did not differ significantly from those obtained when the dose was given in one treatment. Pupal incubation at different temperatures for two hours or two days did not affect egg hatchability in the non-irradiated group. On the other hand, pupal incubation at both 5° or 15°C either for two hours or two days prior to irradiation resulted generally in a significant reduction in the percentages of sterility compared with 25°C. Practically, there was no effect on male survival of either the temperature or the incubation period to which the pupae were exposed prior to irradiation. However, the male competitiveness was better when the pupae were incubated at 5° or 15°C for two days rather than for two hours.

INTRODUCTION

The present experiments are concerned mainly with some factors which were believed to influence the degree of sterility, male survival and competitiveness of the irradiated male medfly. Such factors include the type of larval diet used for rearing the fly in the laboratory, the dose rate of the radiation source, and the conditions at which the sterilizing dose was applied to the pupae such as dose fractionation and temperature at which the pupae were exposed before irradiation.

MATERIAL AND METHODS

Adult rearing cages and methods of collecting eggs were similar to those previously described by Wakid and Shoukry [1]. Male sterility was assessed from egg hatch data of 10 irradiated males mated with 10 normal females per cage. Male longevity was determined by daily counts and removal of dead males in each cage for the first 3 weeks after emergence. The temperature was kept constant in the laboratory at 25° ± 2°C. From 3 to 5 replicates were used and the adults in each replicate were taken from the same batch of pupae. The expected egg hatch was computed as described by Fried [2].

To investigate the role of larval diet on the susceptibility of medflies to gamma radiation the regularly used rearing medium (the molasses medium) was compared with the two other media; sugar and glucose. The latter two media consisted of the same other components as the molasses medium [3]. Eight-day-old pupae were irradiated with 7, 9 and 11 krad using a gamma cell with a dose rate of 30 rad/s.[1]

To study the effect of dose rate, three different sources of gamma radiation having dose rates of 7, 30 and 97 rad/s were used. At each dose rate, 3 doses (7, 9 and 11 krad) were applied to 8-day-old pupae.

In the third experiment, one group of 8-day-old pupae was irradiated at a dose rate of 30 rad/s with 9 krad given in one fraction. Another group received the same dose but in three fractions at one day interval between each treatment (as 6-, 7- and 8-day-old pupae). The fractionated dose was given at three alternatives (2+3+4 krad, 3+3+3 krad or 4+3+2 krad).

In the last experiment, the effect of temperature before irradiation was studied. For the two hours incubation period specially designed double-walled containers were used. The outer part measured 10 cm in diameter and 7 cm high. The inner one was smaller, measuring about 5 cm in diameter and 7 cm high. The inner space was filled with a mixture of small ice cubes and water to keep the desired temperature inside the inner container. When the temperature was maintained at the desired degree (5° or 15°C) for about half an hour, the experiment was started by enclosing the pupae to be irradiated inside the inner container for two hours, then the double walled container was irradiated. For the two days incubation period, the pupae to be irradiated were kept for the desired period in an incubator at 5° or 15°C. For the control, the pupae were kept at the normal laboratory temperature (25° ± 2°C). After irradiation the pupae were transferred to normal laboratory conditions for emergence.

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[1] 1 rad = 1.00×10^{-2} Gy.

TABLE I. FERTILITY, SURVIVAL AND COMPETITIVENESS OF *Ceratitis capitata* MALES REARED AS LARVAE ON THREE DIFFERENT MEDIA AND EXPOSED AS PUPAE TO 3 DOSES OF GAMMA RADIATION

Dose (krad)	Egg hatch (%)	Survivals (%)	Percentage egg hatch at the ratio 3:1:1[a]	
			Actual	Expected
Molasses medium				
7	12.3	83.3	–	–
9	7.2	76.7	40.5	25.8
11	0.8	76.7	–	–
Controls	81.7	73.3	–	–
Sugar medium				
7	9.9	93.3	–	–
9	4.9	93.3	47.4	23.9
11	1.4	86.7	–	–
Controls	81.0	80.0	–	–
Glucose medium				
7	12.8	100	–	–
9	4.7	100	31.9	26.6
11	2.7	93.3	–	–
Controls	92.4	100	–	–

[a] Irradiated males : normal males : normal females.

RESULTS AND DISCUSSION

Larval diet

The results indicate a highly significant reduction ($p > 0.01$) in the egg hatch of eggs produced at all applied doses and in the three media (Table I). With the increase of radiation dose a noticeable gradual decrease in the percentage of egg hatchability took place. However, a comparison of the results of hatchability in the different media showed no influence in this respect.

The percentages of male survivals were not significantly affected by using any of the three media.

TABLE II. EFFECT OF DOSE RATE ON FERTILITY, SURVIVAL AND COMPETITIVENESS OF *Ceratitis capitata* MALES

Dose (krad)	Egg hatch (%)	Survivals (%)	Percentage egg hatch at the ratio 3:1:1[a]	
			Actual	Expected
7 rad/s				
7	25.4	96.7	–	–
9	11.9	100	46.4	30.1
11	8.6	96.7	–	–
Controls	84.8	93.3	–	–
30 rad/s				
7	22.7	66.7	–	–
9	8.4	96.7	35.4	24.0
11	6.6	96.7	–	–
Controls	70.8	80.0	–	–
97 rad/s				
7	16.5	90.0	–	–
9	2.5	93.3	32.6	24.0
11	1.3	86.7	–	–
Controls	88.4	93.3	–	–

[a] Irradiated males : normal males : normal females.

Results of mating competitiveness of males irradiated as 8-day-old pupae (Table I) showed that the differences in the actual percentages of egg hatchability were statistically insignificant ($p > 0.01$) when using the three media. However, when expressed as a competitiveness value [2], the glucose medium showed the highest value. This means that the competitiveness of 9-krad-irradiated males when caged with normal males and normal females at the ratio of 3 : 1 : 1 did not significantly differ in the case of the three larval diets. However, in all the cases studied, the irradiated males were less competitive than normal ones, as the actual percentages of hatchability were higher than the expected ones.

TABLE III. EFFECT OF DOSE FRACTIONATION ON FERTILITY, SURVIVAL AND COMPETITIVENESS OF *Ceratitis capitata* MALES

Dose fractionation	Egg hatch (%)	Survival (%)	Percentage egg hatch at the ratio 3:1:1[a]	
			Actual	Expected
9 krad[b]	0.7	72.5	44.7	24.0
2+3+4 krad[c]	0.3	82.5	43.6	23.7
3+3+3 krad[c]	0.3	77.5	49.6	23.7
4+3+2 krad[c]	1.4	75.5	71.2	24.5
Controls	93.9	70.0	–	–

[a] Irradiated males : normal males : normal females.

[b] The dose was given in one treatment.

[c] The dose was given in 3 fractions with a one day interval between each treatment.

Dose rate

Results of egg hatchability (Table II) indicate a significant gradual decrease in the percentages of hatchability proportional to the increase in radiation dose. At the lowest two tested dose rates, the differences were significant at 5% level of probability between 7 and 9 krad and at 1% level between 7 and 11 krad. At the third dose rate (96 rad/s) the differences were significant at 1% level between 7 and 9 krad and at 5% level between 7 and 11 krad. On the other hand, the differences between 9 and 11 krad were all insignificant at the three tested dose rates. A gradual decrease in egg hatchability was also observed at each dose with the increase in the dose rate. Within dose rates, however, the decrease in egg hatchability was insignificant except between dose rate 7 and 97 rad/s at both 7 and 11 krad.

No effect was shown on the male survival by differences in either dose rate or in the radiation dose.

The results of male competitiveness irradiated as pupae two days before emergence with 9 krad and at three different dose rates are shown in Table II. The (actual) egg hatchability obtained when irradiated males were caged with normal males and normal females at the ratio of 3 : 1 : 1 was higher than the 'expected' at all tested dose rates. This means that the irradiated males were less competitive than normal males whether irradiation was given from gamma source with 7, 30 or 97 rad/s. The differences between the mating competitiveness at all the dose rates tested were insignificant. However, at the dose rate 97 rad/s,

TABLE IV. EFFECT OF PUPAL EXPOSURE TO DIFFERENT TEMPERATURES PRIOR TO IRRADIATION WITH 9 krad ON FERTILITY, SURVIVAL AND COMPETITIVENESS OF *Ceratitis capitata* MALES

Temperature and time of exposure	Percentage egg hatch		Percentage survivals		Percentage egg hatch at the ratio 3:1:1[a]	
	control	treated	control	treated	Actual	Expected
Two hours before irradiation						
5°C	90.9	9.5	96.7	76.7	63.8	29.8
15°C	91.1	0.8	86.7	76.7	52.3	23.8
Two days before irradiation						
5°C	89.1	17.8	96.7	96.7	58.5	35.6
15°C	88.0	8.4	90.0	96.7	46.9	28.5
Controls[b]	93.9	1.7	66.7	73.3	43.9	24.8

[a] Irradiated males : normal males : normal females.

[b] The pupae were exposed to room temperature (25° ± 2°C) all the time before irradiation.

the irradiated males showed the highest competitiveness value, although giving the lowest egg hatch.

Dose fractionation

Results shown in Table III indicate that neither egg hatchability nor survival of irradiated males were affected by dose fractionation. From the present data it could be concluded that the repair from radiation damage due to dose fractionation did not occur when pupae of the medfly were treated with a dose of 9 krad. Also, the changing of the sequence of fractionation from 2+3+4 krad to 3+3+3 krad or 4+3+2 krad revealed insignificant differences. The same conclusion was arrived at by Shoukry [4] and by Mayas [5].

Results of mating competitiveness of males irradiated as 8-day-old pupae with 9 krad given the whole dose once or in three fractions are shown in Table III. The results indicate that the 'actual' percentage of egg hatchability at a ratio of 3 : 1 : 1 (treated males : normal males : normal females) were higher than the 'expected' in all cases. This means that irradiated males were less competitive than normal males even when they were given the dose in fractions, and that there was no effect of fractionation on the male competitiveness, except when the fractionation was 4+3+2 krad where competitiveness was lower than in the other cases. This may have been because the pupae were irradiated at an early age (6-day-old) with 4 krad.

Temperature before irradiation

Pupal incubation at different temperatures for two hours or two days did not affect egg hatchability in the non-irradiated pupae. On the other hand, egg hatchability in 9-krad-irradiated pupae was statistically influenced by both temperature and incubation time. In the case of the two hours incubation period significant reduction in egg hatchability was observed between 5° and 15°C at the level of 1% probability, and there were insignificant differences between 15°C and the control. Incubation of pupae for two days revealed also significant differences in egg hatchability between 5° and 15°C and between these temperatures and the controls at the 1% level of probability. Within the different incubation periods also significant differences were observed between the percentages of egg hatchability at 5° and 15°C at the 5% level of probability (Table IV).

It is evident from Table IV that pupal incubation either for two hours or two days did not affect the percentage of male survival.

For the competitiveness results, it was found from Table IV that the 'actual' percentage of egg hatch obtained at the ratio 3 : 1 : 1 was higher than the 'expected' percentage in all treatments. However, the data showed that the

competitiveness was better when the pupae were incubated at 5° or 15°C for two days than when incubated for two hours.

Langley and Maly [6], after exposing medfly pupae to 4° and 25°C before irradiation with 10 or 30 krad, concluded that total sterility could be obtained by combining cooling and irradiation. On the other hand, Serghiou [7], after irradiating pupae of the same species with 9 krad and exposing adults upon emergence to 6°C for 0, 8, 16, 24 or 36 hours, found that male competitiveness at a ratio of 1 : 1 : 1 decreased with the increase of exposure time to chilling.

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STERILITY INDUCTION; COMPUTER SIMULATIONS

Session 7

THE STERILE MALE APPROACH TO THE CONTROL OF THE SPRUCE BUDWORM, *Choristoneura fumiferana*

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Abstract

THE STERILE MALE APPROACH TO THE CONTROL OF THE SPRUCE BUDWORM, *Choristoneura fumiferana.*

Induction of dominant sterility by irradiation of male pupae or adults of *Choristoneura fumiferana* with 25–30 kR of ^{60}Co irradiation reduced mating competitiveness and longevity. In laboratory populations, release ratios in excess of 10 sterile to 1 normal male were required to achieve even 80% reduction in fertility. Adult males sterilized with Thiotepa remained fully viable in the laboratory, and were very competitive, making 40% more first matings when tested against normal males. Release ratios of 5 sterile to 1 normal male reduced fertility of laboratory populations by 95%. However, adverse effects of Thiotepa treatment reduced the males' ability to respond to females and copulate successfully in the field. Treatment of males with 5–10 kR of ^{60}Co irradiation reduced fertility by 12 to 75%, depending on the stage treated. Eggs that hatched produced larvae with extensive chromosomal aberrations, resulting in delayed inherited semi-sterility. Introduction of males irradiated with 5–10 kR into caged laboratory populations reduced fertility for up to three generations, after which recovery occurs at the lower dose. Additional effects of inherited semi-sterility included sex-ratios biased towards males by 2 or 3:1, reduced larval survival, full competitiveness of adult male offspring, and males and females were equally affected. Field testing of this approach is planned. Pheromone trapping was used to study dispersal of released insects, rate and pattern of emergence of field adults, and adult population density. Released males were detected in pheromone traps even when their numbers were low compared with native population levels. Dispersal of field-collected, laboratory-reared, irradiated or genetically marked males has been compared to determine effects of origin, treatment or phenotypic differences on the ability of released males to perform effectively in the field. Pheromone trapping methods are also being developed to monitor field emergence and predict when releases should begin.

As this is the first report in this Symposium that deals with the use of SIT against a forest insect pest, I will begin with a brief introduction to the insect concerned. The spruce budworm, *Choristoneura fumiferana*, is the most damaging lepidopteran forest insect pest in the north eastern area of north America. It causes serious economic damage to the balsam spruce forests of the

Canadian provinces of Newfoundland, Nova Scotia, New Brunswick, Quebec and Ontario, as well as adjacent states in the United States of America. The budworm is a native pest, whose cyclical abundance can be plotted from forest management records over the past 100 years. An approximate 30-year cycle of peak abundance occurs in unmanaged forests. Estimates of endemic level population densities are somewhat uncertain because of sampling problems, but fall in the range of 100 to 5000 adults per hectare. At epidemic levels, larval densities can reach 1000 per square metre of foliage, and estimates of adult moth densities of from 100 000 to 1 000 000 per hectare would be very conservative.

The dynamics of transition from endemic to epidemic levels are not clearly understood though favourable weather and tree age and condition are important driving variables. For epidemics covering millions of hectares and involving literally billions of insects, large-scale insecticide spraying is at present the only viable alternative for protecting trees until harvest is possible. Improved management and harvesting practices can certainly help reduce our dependence on chemical insecticides, as can the search for alternative control methods including bacteria, viruses, fungi, protozoa and Insect Growth Regulators. However, at present only chemical insecticides and *Bacillus thuringiensis* are registered for operational control.

There is evidence that outbreaks originate from relatively small, localized areas, and subsequently spread to cover large areas. This suggests that early suppression of population increase in these smaller areas could avoid, or at least modify, the development of large-scale epidemics. At low pre-outbreak levels, methods are needed that are most efficient when applied to low target insect densities. Among those available, the Canadian Forestry Service has been investigating the potential of genetic control to suppress incipient outbreaks. The advantages of such an approach include the 40 years base of spruce budworm research, the commercial availability of the sex pheromone for trapping and survey, the availability of mass rearing facilities and, of course, the species-specificity of the method. Potential impediments to successful use of SIT include the facts that the spruce budworm is a native, very well adapted insect, it has a very wide distribution, and undergoes dispersal flights from high population areas that can cover in excess of 100 km under favourable wind conditions.

On balance, there seemed good reason to explore the use of SIT against this insect, if for no other reason than that the information to be gained would prove valuable in applying SIT to other forest insect pests with perhaps greater inherent potential for success.

The SIT approach for control of the spruce budworm has been investigated from two aspects: direct dominant sterility, and inherited semi-sterility. At each step in the investigations, effects of treatment on laboratory and field behaviour have also been examined.

Adult males sterilized by topic application of Thiotepa [1] remain fully competitive, and in fact have a mating advantage over normal males when competing for virgin females. Release ratios of 5 chemosterilized males : 1 normal male : 1 normal female in caged laboratory populations reduced population fertility by up to 90%. However, this effect does not transfer to field situations, where the adverse effects of the chemosterilant on longevity and ability to respond appropriately to the female sex pheromone minimize the effectiveness of releases. No detectable reduction in fertility was found in single caged tree populations at release rates up to 10 sterile to 1 normale male. Chemosterilants do not appear to be an operable option for the spruce budworm.

Irradiation-induced full sterility in male spruce budworm requires doses in the range of 30 kR [2]. At this level, male longevity and mating competitiveness are significantly reduced, and release ratios in excess of 10:1 result in only 80% reduction of fertility of caged laboratory populations. However, as noted with other Lepidoptera [3], substerilizing doses still reduce male fertility, with the benefit that adverse side effects of treatment are minimized. In the spruce budworm 5–10 kR reduce male fertility by 40–60%, and eggs that do hatch produce larvae with extensive chromosome aberrations that impose a high degree of sterility in subsequent gametogenesis [4]. Additional benefits of this lower level irradiation include: (1) F_1 sex ratios biased towards males by 2 or 3 to 1; (2) reduced larval survival; (3) full competitiveness of F_1 adult males; (4) fertilities of irradiated and F_1 females are equally if not more affected.

Release into caged laboratory populations at ratios of 5 irradiated males : 1 normal male : 1 normal female reduces egg hatch and larval survival for up to three generations, after which recovery of fertility occurs in the few survivors.

On the basis of these laboratory findings, induction of inherited semi-sterility in spruce budworm males by substerilizing doses of irradiation appears to be a promising method of reducing population fertility. However, field testing has not yet been carried out, so the real potential of this method against endemic field populations has yet to be established.

As M.D. Proverbs has so correctly pointed out at this and earlier symposia, information additional to the sterilizing method to be used must be gathered if tests of SIT are to be reliable indicators of the potential for a particular insect. For the spruce budworm, we have concentrated to date on effects of treatment on field behaviour of released males, dispersal in both time and place, detection of released males, monitoring of field emergence, and prediction of field emergence for estimating release times. In these studies, we have used pheromone trapping, as it provides a very sensitive method of sampling, as well as a general indication of male response to pheromone sources, the first step in mating.

Release of treated and untreated males, marked either genetically or with fluorescent dyes, and subsequent recapture in pheromone traps, has permitted

us to compare a number of treatments and insect stocks for their potential use in release programmes. Males from laboratory stocks were recaptured in similar numbers to those reared from field-collected larvae. Low-level irradiation had no detectable effect on the ability of males to respond to pheromone and therefore be captured in the traps. Males from a genetic stock carrying an orange eye gene instead of the wild type vermilion were not recaptured at all. We subsequently determined that an altered phototropic response associated with the eye colour mutation was responsible for the failure of the mutant males to be retrapped.

On the basis of these tests, we have concluded that laboratory-bred males marked with fluorescent dusts are sufficiently similar to wild males in behaviour to allow their use in field release studies.

Irradiated marked males appear to disperse relatively uniformly throughout the native population in the area of release. Males were released from ten randomly chosen sites within a one hectare plot. On the next two successive nights, 25 pheromone traps were set out in a square grid pattern. Proportions of marked among total males captured did not vary significantly among traps, indicating a relatively uniform dispersion of males throughout the plot.

In three replicate one hectare plots, we have monitored insect development using conventional foliage sampling techniques as well as pheromone trapping to detect first emergence of males. On 25 June 1979, we detected the first pupae in the foliage samples, indicating the onset of pupation.

The first adult males were captured in traps on 30 June. Trap catches remained uniformly very low until 6–8 July, when rapidly increasing catches reflected the emergence of males. The subsequent cumulative trap catch curves resembled those expected from known budworm emergence patterns. Interestingly enough, the accumulated degree days between the detection of first pupation and detection of first flight equalled 125, quite similar to the value obtained by other workers for the development of pupae under field conditions. This relationship was confirmed in 1980 trials, indicating time of first pupation, accumulated degree days and first male flight might be used to predict field emergence and therefore the time when release would be necessary.

In conclusion, the use of inherited semi-sterility would seem to have potential for reduction of population fertility in the spruce budworm. However, its effectiveness against such a widely distributed insect will have to await assessment in carefully conducted field trials. Use of pheromone trapping to monitor release and dispersal, as well as field emergence, appears to be a valuable tool. Even if SIT should not prove to be effective against the spruce bedworm, even partial success would indicate good potential for SIT against forest Lepidoptera with more restricted population densities and distributions.

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MATING COMPETITIVENESS AND EFFECTIVE LIFE OF THE RADIATION-STERILIZED MALE RED PALM WEEVIL, *Rhynchophorus ferrugineus* Oliv.

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Abstract

MATING COMPETITIVENESS AND EFFECTIVE LIFE OF THE RADIATION-STERILIZED MALE RED PALM WEEVIL *(Rhynchophorus ferrugineus* Oliv.).

The feasibility of using the sterile insect technique for controlling the red palm weevil, *Rhynchophorus ferrugineus* Oliv., a serious pest of coconut and other cultivated palms in India, is being evaluated. In this insect an X- or gamma-ray dose of 1.5 krad induces about 90% sterility in males, who survive for 90–100 days compared with 100–110 days of control insects. Since the sterile males would survive for such a long period after their release in the field it was of interest to evaluate their mating competitiveness as they advance in age in order to determine their effective life. Data from sex ratio experiments indicated that mating competitiveness is reduced by about 50% by the time the sterile male is half way through its life expectancy. Reduction in the overall competitiveness could not be attributed only to the reduction in their mating ability but also to depletion in sperm production during aging. Results are discussed in the light of the precedence in sperm utilization in this insect.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* Oliv., is a serious pest of coconut *(Cocos mucifera)* and other cultivated palms in India [1, 2], Sri Lanka [3], Indonesia [4] and the Philippines [5]. Timely action for the control of this pest is made difficult by the absence of any visual symptoms of infestation. By the time the insect attack becomes discernible irreparable damage has already been done. We are evaluating the feasibility of controlling this pest by the Sterile Insect Release Method (SIRM). Our earlier studies [6] have shown that nearly 90% sterility could be induced in males by X- or gamma irradiation of newly emerged adults with a dose of 1.5 krad.[1] The irradiated males survive for

[1] 1 rad = 1.00×10^{-2} Gy.

90–100 days compared with 100–110 days of the unirradiated ones. Females survive for the same period, their oviposition period ranging from 50 to 60 days. About 75% of the total eggs laid are deposited in the first 30 days, 15% in the next 20 days and the remaining ones afterwards with considerable irregularity.

Mating competitiveness of sterile males is conventionally evaluated on the basis of expected and observed egg hatch when sterile and normal males in varying ratios are caged with a virgin female. The competitiveness value thus evaluated would help to determine the number of sterile males required for release [7]. It is logical to assume that the sterile males after their release would compete, while they survive, with the native normal males. However, when survival of sterile males is longer than the oviposition period of the female, as in the present case, the conventional approach would not yield adequate information on their mating competitiveness. We therefore evaluated the influence of aging on mating competitiveness of sterile red palm weevil males in order to determine their effective life.

MATERIALS AND METHODS

Insects used in this study were reared in the laboratory at 29° ± 1°C following the method described previously [8].

All the experiments were carried out at a temperature of 29 ± 1°C and a relative humidity of 70 ± 5%.

One-day-old males were irradiated in a ^{60}Co source (gamma cell 200) with a sterilizing dose of 1.5 krad delivered at 925 rad/min.

To determine the influence of aging on mating competitiveness, six irradiated males were caged with a normal male and female. At the end of 30, 40, 50 and 60 days the normal pair was replaced with a new freshly emerged male and female. In both cases normal male and female had no previous mating experience. Single pairs of normal male and female and sterile male and normal female were simultaneously kept to serve as controls. Eggs were harvested on alternate days, kept on moist towelling in Petri plates, and their hatching was recorded after six days. Eggs normally hatch in four days at the experimental temperature. Data on fecundity and fertility were recorded.

For evaluating the mating ability of aging sterile males, another set of six irradiated males (1.5 krad) were caged with a normal male and female. At the end of 30, 40 and 50 days, the sterile males were removed and paired individually with a newly emerged virgin female. Four days later the females were dissected to ascertain the presence of sperm in their spermathecae.

Overall competitiveness of sterile males would depend not only on their ability to mate, but also on their ability to produce and transfer adequate quantities of sperm. However, ultimate competition would be at the level of

TABLE I. EFFECTIVE LIFE OF RADIATION-STERILIZED RED PALM WEEVIL MALES

Male age (days)	Egg hatch (%)	
	Ratio expts[a]	Radiation control
30	31.7	11.06
40	39.8	13.8
50	50.9	10.7
60	85.3	12.7

[a] Based on egg laying over 30 days.

sperm [9]. For quantitative evaluation of the sperm transferred by sterile males on aging, another experiment was performed. In this, irradiated males (1.5 krad) were individually paired with normal virgin females, and at the end of 30, 40 and 50 days, females were replaced with fresh virgin females. Four days later the second set of females were dissected to remove their spermathecae. Each individual spermatheca was placed in a cavity block containing 0.5 ml Belar's saline solution and gently pressed with the help of a tweezer to force out its entire sperm content. Uniform suspension of the sperm in the saline solution was achieved by holding the cavity block for 10 min on a vortex mixer. Aliquots of the sperm suspension were pipetted over both the grids of the haemocytometer. Sperms present in 32 randomly selected squares from the four corner squares of each grid were counted under the microscope. Total volume of the 32 squares was 0.2 mm^3.

RESULTS AND DISCUSSION

Egg hatch data (Table I) revealed that there was progressive decrease in the competitiveness of irradiated males as they grew older. In the case of 30-day-old males, egg hatch was 31.7%, and it progressively increased to 85.3% when males were 60 days old. Mating ability of irradiated males also progressively decreased with advancing age (Fig.1). All the 30-day-old males successfully mated, whereas in the case of 40- and 50-day-old males successful matings were only 61.1 and 33.3% respectively. There was a progressive decrease in the quantity of sperm transferred by the irradiated males on aging (Fig.2), and also a 15.7% reduction in the amount of sperm transferred by the 30-day-old sterile males compared

FIG.1. Effect of aging on mating ability of gamma-radiation-sterilized red palm weevil males.

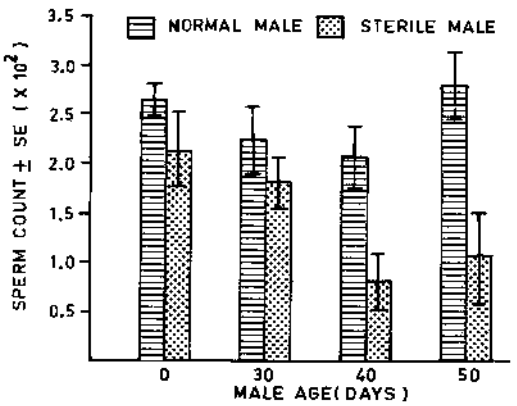

FIG.2. Sperm transfer by normal and irradiated red palm weevil males at different ages.

with controls of the same age. In the case of 40- and 50-day-old males, reduction was about 62%. Decrease in sperm transferred by normal males on aging was, however, not statistically significant. It was difficult to collect sperm quantitatively from the seminal vesicles, therefore sperm content of the spermatheca was considered to be an adequate indication of the sperm production and ability of male to transfer sperm.

Increase in egg viability in the case of 40-day-old males (Table I) could therefore be attributed to their reduced mating ability as well as inadequate transfer of sperm. Reduction in sperm production after irradiation has been

observed in *Heliothes virescens* [10] and *Anthonomos grandis* [11]. Experiments with controlled matings have shown that in this insect sperm transferred during the most recent mating predominantly fertilized the eggs, and after their depletion the sperm transferred during previous mating took part in egg fertilization [6]. Besides the mating competitiveness of sterile males, the nature of sperm precedence in egg fertilization would considerably influence the usefulness of sterile males in the control of insect species whose females are polygamous. In some insects, sperm transferred through multiple matings participate equally in egg fertilization [12, 13] whereas in others the sperm transferred during the second mating are preferentially used [14–19].

It is thus evident from these studies that, although the sterile males survive as long as the normal males, their effective life is only 30 days.

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EVOLUTION OF INSECTICIDE RESISTANCE IN TSETSE

A computer simulation

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Abstract

EVOLUTION OF INSECTICIDE RESISTANCE IN TSETSE: A COMPUTER SIMULATION.

The potential for evolution of insecticide resistance in *Glossina* has been assessed using a combination of computer modelling and chemical analyses. When DDT was topically applied to *Glossina morsitans morsitans*, conversion to DDE was detected at a low level, indicating that flies possess enzymes necessary for resistance to this insecticide. Modelling repeated selection by insecticides on a tsetse population has suggested that the eventual evolution of insecticide resistance is probable if rapid elimination of the treated population is not achieved, provided that the gene for resistance is dominant or co-dominant to its wild-type allele. Immigration is an important factor as it helps prevent population extinction, while diluting the increase of resistant genes only moderately. Other factors influencing the speed of evolution of resistance are: the reproductive fitness of resistant flies, the type of insecticide used (both in terms of its efficiency and persistence) and the regime of insecticide application. Implications for spraying programmes are: (i) the importance of preserving the isolation of treated populations, and (ii) monitoring the insecticide resistance of any remaining flies.

1. INTRODUCTION

In a world-wide survey of resistance in arthropods WHO [1] found that 106 species of medical or veterinary importance had developed resistance to one or more insecticides. A notable exception to the upsurge of resistance in insect pests has been Glossina and to date there are no records of any resistant tsetse populations, in spite of the intensive and long-term spraying operations which have been carried out against tsetse in several African countries.

The generally accepted reason for the absence of insecticide resistance in tsetse is that outlined by Burnett [2] who attributed it to the combination of

low population densities and high susceptibility to insecticides in these flies. However, complete eradication of tsetse populations, although achieved in some areas [3, 4], has by no means been the general rule for control operations, and small populations have occasionally survived even the most intensive spraying programmes[5].

In the absence of field data on the susceptibility of such residual flies, it was decided to re-examine the potential for the evolution of insecticide resistance in tsetse by computer modelling and also by chemical analysis of treated flies.[1]

2. CHEMICAL ANALYSES

This part of the study was restricted to DDT as it has been used for many years in tsetse control and its metabolism in insects is comparatively well understood.

Three groups each of three 1-2-d-old G. morsitans morsitans from the Langford colony were treated by topical application with p,p'-DDT in a solution of di-isobutyl ketone. Three control groups were washed immediately after dosing to determine the amount of DDT applied. The experimental groups were held for 4 h and then washed and extracted to determine the DDT remaining on the outside of the flies and the amount absorbed. The flasks containing the flies were also rinsed for recovery of any DDT on the surfaces on which the flies had rested. Analysis by gas–liquid chromatography showed that a mean amount of 102 ng was received by each fly and, 4 h after application, a mean amount of 68 ng had been absorbed by each fly. Further analysis revealed a mean amount of 1·4 ng DDE in the experimental groups of flies but not in the control, giving a mean conversion rate of internal DDT of 0·7%. Further analysis showed that there was no DDE present in the DDT sample used in the experiment.

Although these results have shown a relatively low conversion rate of DDT to DDE they do nonetheless indicate that tsetse are capable of producing the

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enzyme DDT-ase which is responsible for the breakdown of DDT to DDE and, consequently, many have the potential for the evolution of insecticide resistance.

3. COMPUTER MODELLING

The metabolism of small quantities of DDT to DDE by laboratory-bred flies is in keeping with the enzyme activity expected from susceptible strains of insects. In the wild, natural populations of tsetse may show variation in levels of enzyme production upon which selection by insecticide treatment could act. The computer model was developed to determine whether, and under what circumstances, resistant populations were likely to arise.

A non-overlapping generations model was constructed based on that of Georghiou & Taylor [6], in which the effect of different types of insecticide treatment on both population numbers, and the frequency of a single gene for insecticide resistance, could be tested. Reproduction of the different genotypes was calculated separately (to allow for differential fertility) and was made density dependent so as to stabilize the population at a predetermined size.

Immigration (at 1% of the starting population size) was introduced either before or after reproduction in each generation. The model population could be subject to selection by either persistent (half-life = 50 d) or non-persistent (half-life = 2.6 d) insecticides, at different intensities (initial selection coefficients of 0·999, 0·99 and 0·95). The regimes of treatment were designed to correspond with techniques used in the field with either a single application of a persistent insecticide or sequential applications of a non-persistent type. The resistant mutant was assumed to start at a very low level in the population (frequency = 10^{-5}) and to be either dominant, co-dominant or recessive to its wild-type allele (h = 0·0, 0·5 or 1·0 respectively). The possible effects on reproductive fitness of flies carrying the resistant allele were also studied by reducing the intrinsic rate of increase (r) of resistant flies by 50% in some trials (r was taken as 0·01204 for wild-type flies) [7].

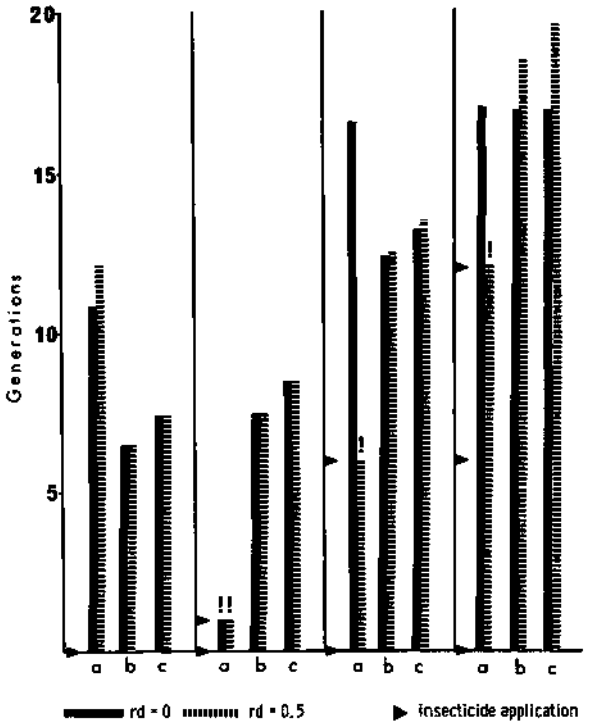

FIG. 1. Computer prediction of time taken in generations for population of tsetse to recover 50% of its original size following different treatments with a persistent insecticide.
a = no immigration
b = 1% immigration/generation before reproduction
c = 1% immigration/generation after reproduction

The effects of a persistent and highly efficient insecticide (s = 0·999), applied at various time intervals, on a simulated population are given in Figs 1 and 2 which show, respectively, the time taken for the population to reach 50% of its original size and the gene frequencies attained at those population levels. Following a single treatment, an isolated population is reduced to very small numbers but not eliminated, whereas two applications made to successive generations result in complete eradication. If the insecticide is applied at yearly intervals then population survival is possible and the frequency of the resistant allele may become very high, unless it is recessive to the wild-type.

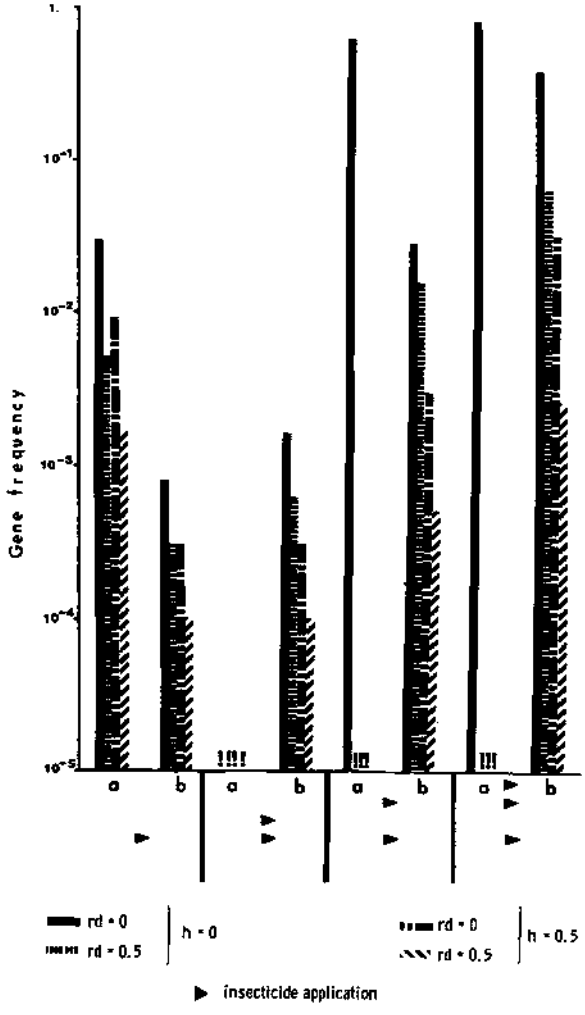

FIG.2. Computer prediction of gene frequencies of resistant alleles achieved when population has recovered 50% of its original size.

a = no immigration

b = 1% immigration/generation before reproduction

The effects of immigration on both fly numbers and gene frequency are illustrated in Figs 1 and 2. While immigration, either before or after reproduction, may result in lower final gene frequencies of the resistant allele, the most important effect is to enable a population to evade eradication even when the mutant is not completely dominant (Fig. 2). Three treatments of a tsetse population at yearly intervals, even with a highly efficient insecticide, could result in very high gene frequencies of resistant alleles in the population given a small amount of immigration. If immigration is prevented then the chances of the population surviving repeated applications are much reduced.

The results have also shown that the degree of dominance is important for the evolution of resistance in tsetse, as completely dominant mutant genes attain higher frequencies in the population than those showing partial dominance. When the resistance allele is completely recessive, much higher initial gene frequencies ($>0{\cdot}001$) are needed for evolution to occur. Since there are no data available on resistance in tsetse, the dominance relationship of such genes cannot be predicted as this may vary between species for any one insecticide [8].

While the magnitude of any loss of reproductive fitness associated with resistance is unknown, results have shown that a reduction in the intrinsic rate of increase of 50% would retard the evolution of resistance and increase the time taken for population recovery. Results obtained with non-persistent insecticides closely parallel those from persistent insecticides and have not therefore been illustrated.

If it is accepted, in view of the present results, that the evolution of insecticide resistance in tsetse has not been prevented by any lack of biochemical competence on the part of the insect, it is remarkable that there have been no reports of resistance following long-term spraying operations. For example, spraying in Nigeria has been in progress since the 1950s and has eradicated tsetse from large areas of the north of the country [9]. The success of this campaign may be partially explained by reference to the present work which has shown that even a single application of insecticide may result in eradication in the absence of immigration. The isolation of treated fly belts

was possible in Northern Nigeria during the dry season when tsetse could be confined to narrow strips of land following river beds [3], and in such circumstances it would be unlikely that resistance would have ever posed a problem.

Control operations in Central and East Africa however have seldom achieved permanent eradication as the fly belts are not normally subject to such severe seasonal restrictions and complete isolation is therefore difficult to maintain [10, 11]. Recently, Davies [5] has suggested that true residual populations remain after treatment with endosulfan both in Zimbabwe [12] and in Botswana [5]. Although these relic populations are thought to have survived due to the increased insecticide tolerance of pregnant females, an alternative explanation would be the evolution of genetically resistant flies. Within the limitations of the highly simplistic model adopted here, it has been shown that insecticide resistance could evolve in tsetse under certain defined circumstances. In particular, the importance of complete isolation during control operations has been demonstrated; failure to prevent immigration could allow populations to evade extinction while subsequent treatments with the same insecticide would encourage the evolution of resistance.

The chemical analyses referred to in this paper were carried out by Dr. F. Barlow (C.O.P.R., Porton Down, U.K.) to whom we are most grateful.

APPENDIX

COMPUTER MODEL

The programme starts with a population of K flies in which the numbers of each genotype are :

$$n_{RR} = p^2K \; ; \; n_{RS} = 2pqK \; ; \; n_{SS} = q^2K$$

where

p = starting frequency of the resistant gene

q = frequency of the susceptible allele (= 1-p)

The new gene frequency in subsequent generations is given by

$$p = (2n_{RR} + n_{RS}) \; / \; 2n_T$$

$$q = (2n_{SS} + n_{RS}) \; / \; 2n_T$$

For each successive generation the numbers of progeny of each genotype are calculated separately as follows

$$m_{RR} = n_{RR} \exp \left[tr_{RR} \cdot (K - n_T) \; / \; K \right]$$

where

m_{RR} = progeny from RR mothers in next generation
n_{RR} = no. of RR flies in present generation
r_{RR} = innate capacity for increase of RR individuals
n_T = total population in present generation ($n_{RR}+n_{RS}+n_{SS}$)
t = generation time (set at 54d)
K = carrying capacity of the environment (set at 10^3)

The numbers of the progeny of the other two genotypes (m_{RS} and m_{SS}) are similarly calculated.

In the following generation the numbers of each genotype (n') are then

$$n'_{RR} = \left[(2n_{RR} + n_{RS}) \; (2m_{RR} + m_{RS}) \right] \; / \; 4n_T$$

$$n'_{RS} = \left[m_{RR}(2n_{SS} + n_{RS}) + m_{SS}(2n_{RR} + n_{RS}) + m_{RS}n_T \right] \; / \; 2n_T$$

$$n'_{SS} = \left[(2n_{SS} + n_{RS}) \; (2m_{SS} + m_{RS}) \right] / \; 4n_T$$

Selection is then applied to the different genotypes as follows

$$n'_{RR} = n_{RR}$$

$$n'_{RS} = n_{RS}\,(1-hs)$$

$$n'_{SS} = n_{SS}\,(1-s)$$

where

n_{RR} = No. of RR individuals before selection

n'_{RR} = No. of RR individuals after selections (and similarly for n_{RS}, n'_{RS}, n_{SS}, n'_{SS})

s = selection coefficient

h = degree of dominance of the wild-type

To model the decline in insecticide efficiency with time, s is computed as a function of time so that

$$s_t = s_0 \exp\left[\frac{-\log_e 2.\ t}{t_{\frac{1}{2}}}\right]$$

where

s_0 = initial selection coefficient

s_t = selection coefficient after t days

$t_{\frac{1}{2}}$ = half-life of insecticide in days

To model a decrease in fitness of 50% associated with the resistant genotype, the value of r, initially 0.01204, is altered according to genotype so that

$$r_{RR} = \tfrac{1}{2}\, r_{SS}$$

$$r_{RS} = \tfrac{1}{2}\, r_{SS}(1+h)$$

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MATHEMATICAL ANALYSIS OF LABORATORY DATA ON THE GROWTH OF TSETSE FLY POPULATIONS

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Abstract

MATHEMATICAL ANALYSIS OF LABORATORY DATA ON THE GROWTH OF TSETSE FLY POPULATIONS.

An attempt is made to establish a quantitative basis for controlling and optimizing productivity in the mass rearing of tsetse flies. The general results obtained for the expanding phase as well as the stationary phase of a colony are illustrated by using data from two colonies of tsetse flies (*Glossina p. palpalis;* the colonies only differ in the feeding technique applied). The following points are taken into consideration: Introduction of adequate age group periods, calculation of fertility rates from productivity and mortality, comparison of the feeding techniques with respect to productivity, approximation of the rate of increase and optimization of productivity by limitation of fly age.

1. INTRODUCTION

In order to cope with the increasing demand for laboratory-reared tsetse flies it is necessary to develop effective quantitative methods to control and optimize productivity in a colony. For this reason a study has been carried out based on so-called control groups. (A control group is a cohort started with a number of females who emerged on the same day; each cohort is regarded as a sample taken at random from the colony.) This investigation first deals with the problem of determining the 'natural' length of an age group period (AGP) and the problem of predicting total productivity from the productivity observed in the early stages. In addition, a comparative study is presented of the feeding techniques applied. On the basis of these results the problem of estimating the rate of increase for expanding colonies is considered and, finally, the question of optimizing productivity by limitation of fly age.

2. EMPIRICAL BACKGROUND

This study is based on data from two colonies of tsetse flies *(Glossina p. palpalis)* reared in the Laboratory of the International Atomic Energy Agency

CAGE NO. K4120- 3

D.ECL. 20. 4.79 D.MAT. 23. 4.79 D.SEP. 25. 4.79 NO.AGP 10

NUMBER OF FEMALES

I	1	2	3	4	5	6	7	8	9	10	I
1	I	15	15	15	15	15	15	15	15	15	15	I
2	I	15	15	15	15	15	15	15	15	15	15	I
3	I	15	15	15	15	15	15	15	15	15	15	I
4	I	15	15	13	13	13	13	13	13	13	13	I
5	I	13	13	13	13	13	13	11	11	10	10	I
6	I	10	10	10	10	10	10	10	10	10	8	I
7	I	6	6	6	6	6	6	6	4	4	3	I
8	I	3	3	3	3	3	3	3	3	2	2	I
9	I	2	2	2	2	2	2	2	2	2	2	I
10	I	2	2	2	2	2	2	1	I	1	1	I

NUMBER OF PUPAE

I	1	2	3	4	5	6	7	8	9	10	I
1	I	0	0	0	0	0	0	0	0	0	0	I
2	I	0	0	0	0	0	0	0	1	4	4	I
3	I	3	0	0	0	0	0	1	3	4	3	I
4	I	0	2	0	0	0	1	2	0	6	2	I
5	I	1	0	0	0	0	0	3	0	2	0	I
6	I	1	0	0	0	0	1	3	0	0	0	I
7	I	0	0	0	0	0	2	0	0	0	0	I
8	I	0	0	0	0	1	1	0	0	0	0	I
9	I	0	0	0	0	1	0	0	0	0	0	I
10	I	0	0	0	0	1	0	0	0	0	0	I

STATISTICS

AGP	I	SURV.	I	FERT.	I	PPF	I	PUP	I	AV.W.	I	SE	I
1	I	100.0	I	0.000	I	0.000	I	0	I	*****	I	******	I
2	I	100.0	I	.600	I	.600	I	9	I	32.74	I	.860	I
3	I	100.0	I	.933	I	1.533	I	14	I	31.88	I	.963	I
4	I	86.7	I	1.000	I	2.400	I	13	I	29.48	I	.749	I
5	I	86.7	I	.462	I	2.800	I	6	I	28.38	I	1.662	I
6	I	66.7	I	.500	I	3.133	I	5	I	29.14	I	1.581	I
7	I	40.0	I	.333	I	3.267	I	2	I	31.20	I	2.200	I
8	I	20.0	I	.667	I	3.400	I	2	I	25.65	I	3.750	I
9	I	13.3	I	.500	I	3.467	I	1	I	27.30	I	******	I
10	I	13.3	I	.500	I	3.533	I	1	I	19.90	I	******	I

PUPAL WEIGHT

AGP	I	WEIGHT
1	I	
2	I	35.5 32.2 31.5 32.3 28.0 36.2 35.4 31.4 32.2
3	I	26.2 31.3 35.2 27.2 30.8 34.8 33.0 25.5 36.0 34.2 36.2 34.0 32.4 29.5
4	I	28.8 25.0 34.1 30.4 28.1 29.6 26.2 30.4 30.2 29.3 31.6 26.2 33.4
5	I	29.6 30.0 32.2 31.4 25.6 21.5
6	I	28.7 35.0 28.8 27.7 25.5
7	I	29.0 33.4
8	I	21.9 29.4
9	I	27.3
10	I	19.9

FIG. 1. Statistics sheet for cage K4120/3.

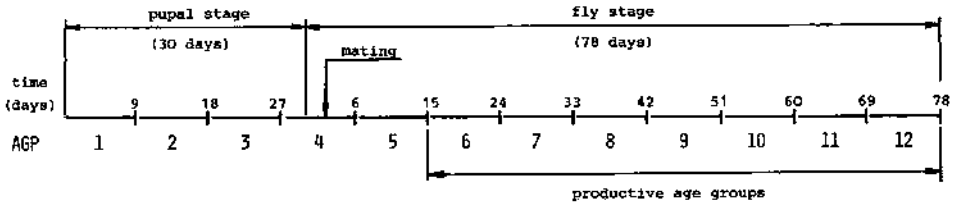

FIG.2. Division of life span into age groups.

(Joint FAO/IAEA Division) in Vienna. According to the production model proposed by Wetzel [1], the colonies are divided into so-called production units, each consisting of a certain number of cages; each cage is started with a cohort of $n_0 = 15$ females. The females are paired at the age of three days with an equal number of males (the same males are used for two other matings). Productivity is at a maximum approximately 30 days after emergence and then declines almost linearly, indicating that the less productive cages should be replaced by new ones. In one colony (colony 1) membrane feeding with defibrinated bovine blood was used and in the other colony (colony 2) membrane feeding with freeze-dried beef blood. From colony 1 a sample of 67 cohorts (cages K1D70-K1F83) was available and from colony 2 a sample of 52 cohorts (cages K4072–K4219).

In order to record the raw data being collected from each cohort in a way that is suitable for further processing by a computer, a specific coding sheet has been introduced. This sheet, one for each cage, contains for each day after emergence the number of females still alive, the number of deposited pupae, the corresponding pupal weights and a cage identification. After having been stored in an adequate file the data are ready for computer-aided evaluation. It is now, of course, no problem to produce also a statistics sheet for the laboratory files (Fig.1).

One of the first questions when constructing an abridged life table is how to divide the life span into age groups. It is suggested that methods of spectral analysis should be applied to find a 'natural' division into age groups [2]. By determining the dominant cycles in the productivity patterns of all cohorts under consideration, it can be concluded that the natural period of productivity for colony 1 as well as colony 2 follows a normal distribution with a mean of 9.1 days and a standard deviation of 0.7 days. As a consequence, the nine-day interval is regarded as the natural length of an AGP and thus a biologically based division of the life span into nine-day cycles is obtained which is presented in Fig.2.

TABLE I. LIFE TABLE PARAMETERS l_j (SURVIVAL RATE) AND m_j (FERTILITY RATE) FOR COLONY 1 AND COLONY 2 *(Glossina p. palpalis)*

AGP-index	Colony 1		Colony 2	
j	l_j	m_j	l_j	m_j
5	0.999	0	0.983	0
6	0.971	0.759	0.967	0.769
7	0.948	0.731	0.932	0.777
8	0.915	0.707	0.871	0.777
9	0.861	0.630	0.792	0.758
10	0.775	0.596	0.682	0.690
11	0.685	0.514	0.553	0.606
12	0.575	0.454	0.410	0.576

Let n_i be the number of females surviving to the i-th day of the fly age (because of the very low mortality at the beginning it is justified to state $n_7 = n_0$). The number of days lived in AGP j by all females of a cage is then given by Σn_i with i running from 7+9 (j−4) to 7+9 (j−3). Division by the AGP-length and rounding gives an integer, called FEM_j, which can be interpreted as the number of fictitious females being alive during the whole length of AGP j. FEM_j is assumed to follow a binomial distribution with some parameter l_j. Because of the natural choice of the age group periods it is possible to describe the deposition of pupae by one female in each AGP j by a Bernoulli variable X_j with $X_j = 1$ indicating 'birth' and $X_j = 0$ 'no birth'. Thus the number PUP_j of pupae deposited in AGP j can be written as the sum of a random number (i.e. FEM_j) of independent, and for each cohort, identically distributed random variables X_j. It follows that with FEM_j being binomially distributed, also PUP_j should follow a binomial distribution with parameter $l_j m_j$ where $m_j = P(X_j = 1)$. (The empirical distribution of PUP_j agrees with the theoretical one only for early age groups, and becomes increasingly dispersed with increasing j; for a discussion of this point see Ref. [2].) Accordingly the formula $E(FEM_j) = n_0 l_j$ and the formula $E(PUP_j) = n_0 l_j m_j$ are used to calculate the AGP-specific survival rate l_j and the AGP-specific fertility rate m_j. Both rates are summarized in Table I. Attention should be paid to the normalization of the survival rate: l_j expresses the fraction of females surviving from emergence to approximately the middle of AGP j. If l_j is multiplied by E, i.e. by the fraction of females surviving from deposition to emergence, another possible normalization is obtained (throughout this study E was assumed to be 0.96).

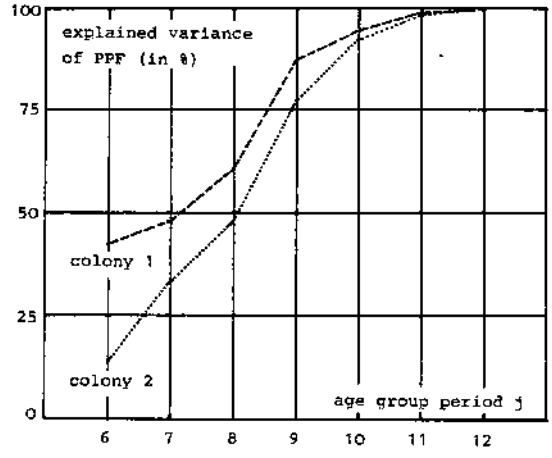

FIG.3. Increase in explained variance of PPF (in percentage of total variance) by contributions up to AGP j.

3. DISCUSSION OF PRODUCTIVITY

A remarkable difference between colony 1 and colony 2 is shown in Table I in so far as the fertility rate is higher by using membrane feeding with defibrinated bovine blood rather than with freeze-dried beef blood. The survival rate, however, shows the opposite behaviour leading to an essentially equal productivity in both colonies. A common measure of the total productivity of a cohort is the PPF-value which expresses the average number of pupae deposited in a cohort per female up to the last AGP, i.e. AGP 12. This quantity can be assumed to follow a normal distribution with a mean of 3.73 and a variance of 0.72 in colony 1 and a mean of 3.80 and a variance of 0.39 in colony 2. Thus no difference in the mean is to be expected between cages fed with defibrinated bovine blood and those fed with freeze-dried beef blood, but there is a significant difference in the variance, mainly caused by the high correlation between the PUP_j of different age groups in colony 1. This point of view is confirmed by Fig.3 which shows the increase of explained variance of PPF by adding an increasing number of age group periods. The difference between both colonies is very striking in the early age group periods and it is seen that a prediction of PPF with, for example, PUP_6, only makes sense in the case of colony 1. Both colonies are, however, characterized by an outstanding jump in the explained variance between AGP 8 and 9 so that the whole productivity can be thought of as the result of two 'forces of productivity', one acting in AGP 6–8, the other in AGP 9–12.

The intrinsic rate r of natural increase is probably the most important quantity in the expanding phase of a colony. It is desirable for practical reasons to have a formula which allows easy computation of r as well as revealing the relation between r and other life history parameters, e.g. the net reproduction rate R_0 which is essentially the same as the PPF-value. It is customary in ecology to make use of the approximation $r \approx r_c = \ln R_0/\mu$ where μ expresses the mean age of producing offspring [3, 4]. Consider for example the survivorship and fecundity schedules as given by Table I for colony 1. Using the formulae (φ is the fraction of female pupae)

$$R_0 = E\varphi \sum_{j=6}^{12} l_j m_j, \quad R_1 = E\varphi \sum_{j=0}^{12} (j+0.5)\, l_j m_j \tag{1}$$

and $\mu = R_1/R_0$, the intrinsic rate of natural increase is found to be $r_c = 0.00983$ 1/d (d = day). A better result, i.e. a result which is nearer to the exact value $r = 0.01029$ 1/d, is obtained by using (according to Ref. [5]) the formula

$$r \approx r_e = -\frac{R_1}{R_2} \ln \left[1 + \frac{R_2}{R_1^2}(1 - R_0) \right] \tag{2}$$

with R_0 and R_1 as defined in formula (1) and

$$R_2 = E\varphi \sum_{j=6}^{12} (j+0.5)^2\, l_j m_j$$

Approximation (2) leads to $r_e = 0.01010$ 1/d for colony 1. It was mentioned above that the productivity pattern has a dominant cycle with a period of nine days. Of course, this cyclic behaviour is not expressed by the AGP-specific fertility rates in Table I. It can be shown that neglect of the cyclic behaviour of the productivity pattern results in an overestimation of r by about 10% [5]. Thus, in the case of colony 1, the intrinsic rate of natural increase is expected to be $0.9r_e = 0.00919$ 1/d rather than r_e.

In the stationary phase the intrinsic rate of natural increase is zero, and the most important question is now how many male offspring can be removed from the colony without affecting the size of the colony and how this number can be made as large as possible. Let SM be the number of sterile males available, i.e. not required for the maintenance of the colony, per unit time. If this number is related to the number of cages one obtains the quantity $SM/C(\omega,u)$ as a useful index of productivity. This index depends on the

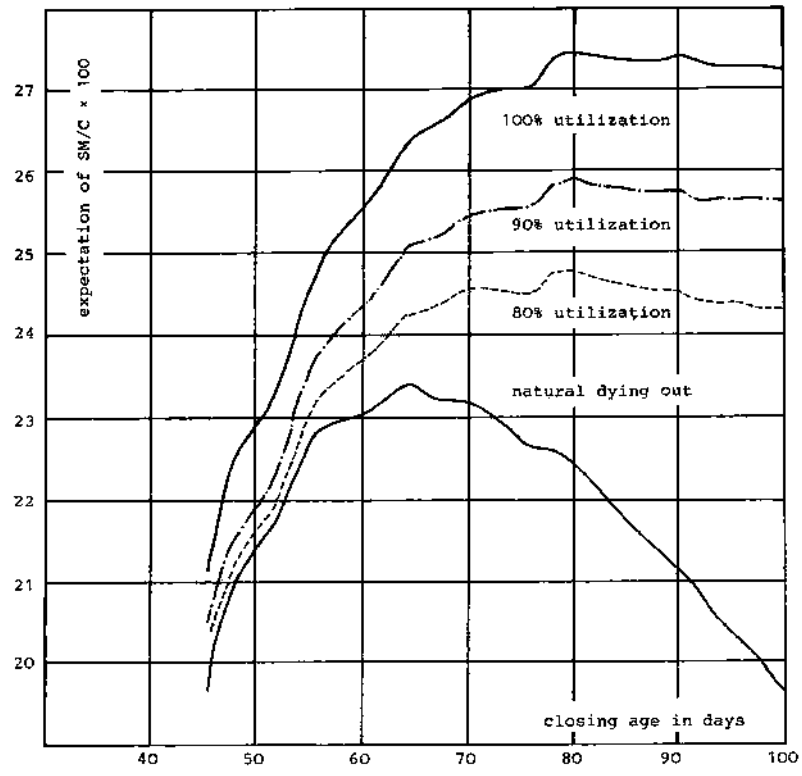

FIG.4. Expected number of males available for release per cage and day (colony 1).

maximum attainable age ω (in days) of the flies in the colony as well as on the degree u of utilization of the cages (for fixed age defined as the average number of females per cage divided by n_0). A lower boundary for u is given when the cages die out in a natural way. An increase of u above this boundary (up to 100%) is only possible if flies from different cages are combined. It can be concluded for both colonies that SM/C(ω,u) is a random variable following a normal distribution for all numbers ω and u of interest. The expectation of SM/C depends, of course, on ω and u as illustrated by Fig.4 for colony 1. It is seen, for example, that in the case of natural dying out the optimal value $SM/C_{opt} = 0.233$ is obtained at $\omega_0 = 64$d, whereas at $\omega = 100$d only 0.197 sterile males are available per cage and day. This means that by reducing the fly age from 100 to 64 days the productivity is increased by about 18%. If males are only used for one rather than three matings the index SM/C is considerably reduced (approximately by a factor 1/2), and in addition the optimal closing age ω_0 is shifted towards the end of the fly stage. A detailed discussion of the formulae used to calculate the maximum of SM/C is given in Ref. [6].

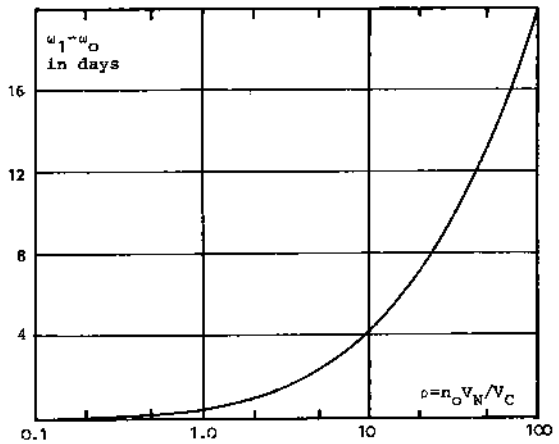

FIG. 5. Optimal closing age ω_1 as a function of the cost ratio $\rho = n_0 V_N/V_C$

It is also possible to write down an explicit expression for the optimal closing age ω_0 if only the case of natural dying out is considered and the average number p(i) of offspring borne by one female on day i is assumed to be linearly declining, i.e. $p(i) \approx p(\alpha) + K\alpha\text{-}Ki$ (α indicates the beginning of the reproductive period within the fly stage). Then

$$\omega_0 = \sqrt{\alpha^2 + \frac{2}{K}\left[p(\alpha)\,\alpha + \frac{1}{bs}\right]} \tag{3}$$

where s is the sex ratio between male and female pupae and b the average number of females being mated by one male [5]. For colony 1 of Table I the constants needed in (3) are: $\alpha = 16$d, $s = 0.44/0.56 = 0.79$, $K = 0.00058\ d^{-1}$, $p(\alpha) = 0.0506$. If $b = 2.56$, then $\omega_0 = 68$d follows from formula (3). The corresponding maximum male surplus per cage and unit time is given by

$$SM/C_{opt} = \frac{n_0}{\omega_0}\left(\frac{s(\omega_0-\alpha)}{2}\,[2p(\alpha) - K(\omega_0-\alpha)] - \frac{1}{b}\right)$$
$$= 0.0156\, n_0$$

If $n_0 = 15$ then $SM/C_{opt} = 0.234$ which agrees very well with Fig.4. It is again emphasized that a sufficiently large b is essential for getting a high SM/C_{opt}. For example, $b = 1$ would result in $\omega_0 = 86$d and $SM/C_{opt} = 0.116$, which confirms the assertion made before that productivity is lowered by 50% in this case.

The optimization problem previously considered is now generalized by introducing a cost function V. It seems to be reasonable to write V as $V = V_0 + V_C C + V_N N_e$ where V_0, V_C and V_N are suitably chosen constants. C is the number of cages in the colony and N_e the number of females emerging per unit time. By splitting V up into the fixed cost term V_0 and the term $V_C C$ as well as $V_N N_e$, it is assumed that the number of cages and the number of females emerging per unit time (or the number of pupae deposited per unit time) are the most relevant quantities that influence the total cost of running a colony. In order to make the cost per sterile male as low as possible we have to minimize the expression

$$V/SM = V_0/SM + V_C \frac{C}{SM} + V_N \frac{N_e}{SM}$$

where SM is the number of sterile males available per unit time. It is shown in the Appendix that V/SM is at a minimum if the fly age is limited to ω_1 as given by

$$\omega_1 = -\rho + \sqrt{(\alpha+\rho)^2 + \frac{2}{K}\left[p(\alpha)(\alpha+\rho) + \frac{1}{bs}\right]} \qquad (4)$$

where $\rho = n_0 V_N/V_C$. For $\rho = 0$ the optimal closing age ω_1 coincides with ω_0. If $\rho > 0$ then $\omega_1 > \omega_0$, and Fig.5 shows how ω_1 depends on ρ.

APPENDIX

DERIVATION OF FORMULA (4)

Consider a colony being stationary at a certain size N (N = number of females in the colony). The total number of female pupae deposited per unit time follows from $B_f = NR_0/(L_\omega E)$ where E is the emergence rate and L_ω the average number of days spent by one female in the fly stage (being limited by age ω). R_0 is the net reproduction rate given by

$$R_0 = \sum_{i=\alpha}^{\omega} p(i) \approx \int_{\alpha}^{\omega} p(i)\, di$$

with $p(i) = l_i m_i$ and l_i and m_i being the daily survival and fertility rate respectively. In order to keep the size of the colony at a constant level, only B_f/R_0 female pupae are needed from which $N_e = B_f E/R_0 = N/L_\omega$ females emerge per unit time. The number of males necessary for mating these N_e females per unit time is given by $M = N_e/b$ where b is the average number of females that are mated by one male. Finally, the number of cages in the colony is given by $C = N_e \omega/n_0$ provided that only the case of natural dying out is taken into consideration. Thus

$$SM = sB_f E - M = \left(sR_0 - \frac{1}{b}\right)N_e$$

sterile males are available per unit time (s is the sex ratio between males and females). Taking $Z = (V - V_0)/SM$ as the new cost function, we now have to minimize

$$Z(\omega) = V_C \frac{C}{SM} + V_N \frac{N_e}{SM}$$

$$= \frac{V_C}{n_0} \frac{\omega + \rho}{sR_0 - \frac{1}{b}}$$

with respect to ω. From $dZ/d\omega = 0$ it follows that

$$s \int_{\alpha}^{\omega} p(i)\, di = \frac{1}{b} + (\omega + \rho)\, p(\omega)\, s$$

as a necessary condition for some ω in order to be a minimum point of $Z(\omega)$. In addition it is easily shown that the second derivative of $Z(\omega)$ is always positive since $dp/di < 0$. By using the linear approximation $p(\alpha) + K\alpha - Ki$ for $p(i)$, formula (4) is finally obtained.

ACKNOWLEDGEMENTS

I wish to thank H. Wetzel and the staff of the Seibersdorf Laboratory, in particular D. Luger, for their co-operation. I also wish to express my gratitude to G. LaBrecque of the Joint FAO/IAEA Division for his assistance.

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POSTER PRESENTATIONS

Session 7a

Poster Presentations

MASS REARING OF THE OLIVE FRUIT FLY
Recent improvements

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The evolution of the mass rearing system for the olive fruit fly, *Dacus oleae* (Gmel.), has been described [1–3]. An improved system, applied at present at the Demokritos Nuclear Research Centre, can produce one to one and a half million insects, in laboratory level conditions, at a production cost of 17 drachma/1000 pupae (50 drachma = 1 US$). This is about half the cost calculated in 1977 [3], despite considerable increases in material and wages costs which have occurred in the meantime.

The marked cost reduction has been achieved through improvement in the adult diet, the use of a new larval diet tray and the mechanization of certain phases in the rearing system. The new adult diet is composed of sugar, yeast hydrolysate and dry egg yolk at a ratio of 80:30:6.6, and it differs from the one previously used [2] in the amount of yeast hydrolysate (a ratio of 20 previously) and dry egg yolk instead of fresh yolk. The egg production on the new diet is significantly higher (by about 20%) than the previous one. A rectangular plastic tray (48 × 38 × 2 cm holding 1250 g diet) gave a yield of 5.4 pupae/g diet compared with 2.93 pupae/g diet [3] which was the production of the previously used round trays (dia. 32 cm, height 0.8 cm, holding 230 g diet). These values have been calculated on the basis of a production of several million pupae. To homogenize larval diet to a uniform granular form, previously work performed manually [3], a mechanical device was developed. It consists of a stationary horizontal cylinder (dia. 32 cm, height 65.5 cm) of aluminium screen (stretched metal with diamond-shape openings 12 × 6 mm) with a stainless-steel axial scraper. The revolving scraper forces the diet through the openings of the side of the cylinder, achieving in this way a uniform granular consistency. The separation of pupae from the sawdust, serving as pupation medium, instead of manual sifting is done with a mechanical sieve moving reciprocally at a frequency of 210 laps/min. Sifting of first-day pupae for up to two minutes, second-day pupae for up to four minutes, and for 15 minutes for older pupae did not adversely affect adult emergence. A possible adverse effect on the flight capabilities of flies sifted as pupae, as was found with other

TABLE I. PRODUCTION COST OF THE OLIVE FRUIT FLY, BASED ON A DAILY PRODUCTION OF 224 000 PUPAE

Cost constituent	Man-hours	Cost (drachma)
Larval diet	–	2531.20
Adult diet	–	23.30
Larval diet labour	1543	154.30
Colony labour	4833	483.30
Miscellaneous labour	2833	283.30
Colony insects	–	110.00
Miscellaneous	–	222.60
Total	9209	3808.00

Cost of 1000 pupae, 3808/224 = 17 drachma (50 drachma = 1 US$).

tephritids [4], has not been investigated. The introduction of the two mechanical devices reduced labour by about 90%.

Under production conditions of half a million flies per week in the summer of 1980, last instar larvae were infected by *Pseudomonas aeruginosa*[1], a potential insect pathogen under adverse environmental conditions. At peak infection, pupal mortality reached the 70% level. It seems that stress conditions caused the severe infection. By decreasing the rearing temperature by 2 degC, making up for the increase of temperature due to metabolic heat of the last larval instar, and increasing ventilation of the diet environment, which reduced ambient relative humidity also, the problem practically disappeared.

A concise estimate of the olive fruit fly production cost, considering only expendables and labour, is shown in Table I. From 40 adult cages a daily egg production of 640 000 is expected to produce 224 000 pupae (35% eggs). To produce these pupae, 41.5 kg larval diet is required.

[1] Thanks are due to G. Thomas of the Department of Entomological Sciences, University of California at Berkeley, for the identification of the bacterium.

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SEXUAL BEHAVIOUR OF THE OLIVE FRUIT FLY, *Dacus oleae*

Some differences between wild and laboratory-reared flies

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Some aspects of the sexual behaviour of wild and artificially reared olive flies were compared in the laboratories at the Demokritos N.R.C. during 1976–80.

The results are as follows:

1. SEXUAL AND REPRODUCTIVE MATURATION

Laboratory-cultured olive flies become sexually mature (when mating is the criterion of sexual maturation) from the 3rd to the 5th day after adult emergence. Radiation of lab-flies [1] with 11 krad of gamma rays, in an N_2-atmosphere, 1–3 days before adult emergence [2], delays sexual maturation by 1–2 days.[1] No differences were found between three lab-strains of flies which had been artificially reared for 120, 60 and 10 generations respectively.

Wild flies, collected from infested fruit, become sexually mature much later than lab-flies. They mature from the 4th to 15th day after adult emergence.

[1] 1 rad = 1.00×10^{-2} Gy.

In all categories of flies tested, males mature 1–2 days earlier than females.

A high maturation rate of the lab-flies, i.e. maturation within the first five days after adult emergence, is obtained when the wild flies have been artificially reared for 3–5 generations.

Studies on ovarian development have shown that in lab-females eggs mature from the 4th to the 6th day after adult emergence, whereas the wild females developed eggs from the 6th to the 17th day.

The first mating occurs mostly in the late stage of the vitelogenesis. Mature eggs in the ovaries are observed 1 to 2 days after mating. Oviposition starts immediately after egg maturation.

2. MATING FREQUENCY IN MALES AND FEMALES

The males of the olive fly can be characterized as polygamous. They are able to mate daily up to 40 consecutive days. The previous mating of the male does not affect its sexual rhythm and activity.

Non-irradiated males, wild or laboratory reared, convey sperm to all their mating partners, whereas irradiated males convey sterile sperm only from the 4th to 14th mating, after which they convey only seminal fluids.

Wild males begin to mate much later, and mate less frequently, than lab-males, normal or irradiated.

Females after each successful mating become sexually unreceptive for several days. The intermating period is longer in wild females than in those artificially reared.

Experiments with single pairs, 1♀+1♂, in small cages, showed that the females are oligogamous. Most females mated 1 to 2 times; considerably fewer mated three times during their life. Among laboratory-reared females, the sterilized ones mate more frequently than the non-sterilized whereas wild females mate less than both categories of artificially reared females.

In the presence of more than one male, one female with two males or groups of both sexes, the females showed a mating frequency 3 to 4 times higher than in experiments with pairs. One explanation is that the presence of more than one male leads to matings with unreceptive females.

3. DISTRIBUTION OF MATINGS WITHIN THE MATING TIME

The mating time of the fly is controlled by a circadian rhythm, and appears during the last hours of the photophase [3, 4].

Experiments in different photoperiods such as LD 4:20, LD 8:16, LD 12:12, LD 16:8, LD 20:4, as well as during the maturation process and in

populations of males to which laboratory virgin females were offered daily for over 40 consecutive days, were found to result in mating peaks of both populations (wild-lab) which differ by 1 to 2 hours. It appears that in the lab-flies the mating rhythm occurs 1 to 2 hours earlier than in wild flies. This difference in the distribution of matings can lead to a partial isolation among the two populations.

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INFLUENCIA DE LA TEMPERATURA MEDIA DIARIA EN EL DESARROLLO DE *Ceratitis capitata* (Wied.) EN AREAS CITRICOLAS

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INTRODUCCION

Una de las plagas que mayor incidencia tienen en el cultivo de los frutales y particularmente cítricos, en España, es *Ceratitis capitata* Wied., debido a los elevados costes de los tratamientos insecticidas obligatorios para mantener un nivel mínimo de daños y por razones de cuarentena.

Dentro del plan de lucha integrada que el Ministerio de Agricultura lleva a cabo en toda la zona citrícola y hasta que el problema de la separación de sexos en la cría masiva de *C. capitata* no esté resuelto para aplicar, sin problemas, el método de los insectos estériles (SIT), se ha efectuado un estudio de la biología de *C. capitata* en esta zona con el fin de determinar el número de generaciones anuales y la duración de cada fase de desarrollo del insecto según la climatología. Los estudios de Bodenheimer[1] no son adecuados para esta zona.

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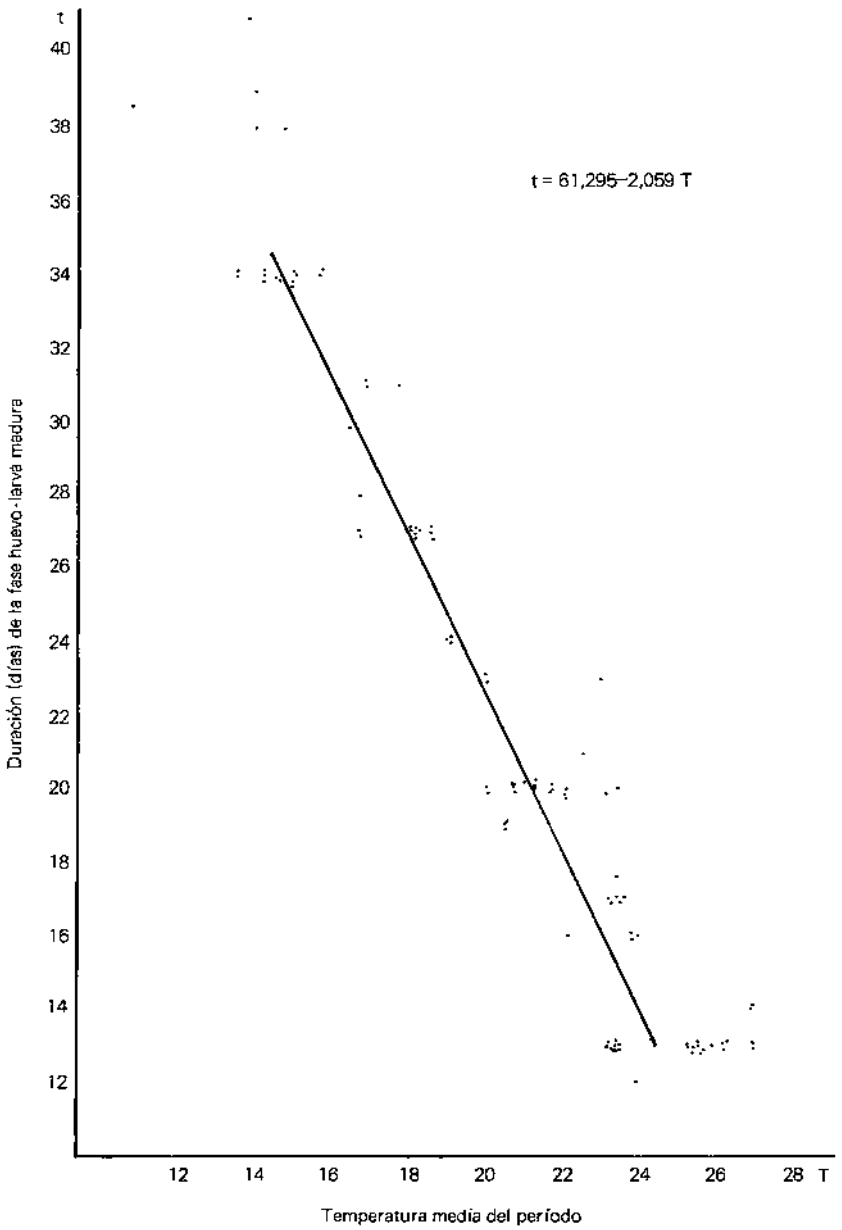

FIG. 1. Período mínimo de la fase huevo-larva madura de C. capitata *Wied. en naranjas.*

MATERIAL Y METODOS

En la provincia de Castellón, en el centro de la gran zona citrícola de España, se cubrió un naranjo con una malla acrílica, en cuyo interior se liberaron moscas fértiles procedentes del laboratorio de cría masiva de *C. capitata* del Instituto Nacional de Investigaciones Agrarias (El Encín), suministrándoles agua y alimento.

Durante todo el año se mantuvo una población de adultos renovados cada quince días. Cada tres días se introducían cinco naranjas a fin de que las hembras pudieran hacer la puesta. Al tercer día se retiraban los frutos, suspendiéndolos en árboles contiguos de forma similar a los naturales.

Diariamente se inspeccionaban los frutos, así como se llevaba el control de la temperatura y humedad mediante un termohidrógrafo situado en el mismo árbol. De esta forma se han obtenido 120 "generaciones" (huevo-adulto) en distintas épocas del año.

Con los datos obtenidos se correlacionó la duración de los períodos de desarrollo huevo-larva y pupa-adulto con las temperaturas medias diarias y con la temperatura media del período de desarrollo.

Sería muy extenso el cuadro correspondiente a las 120 generaciones, basten pues dos ejemplos:

Fecha picadura	Fecha salida larva	Fecha pupa	Fecha eclosión adulto	Temp. período larva	Temp.período pupa
12 julio	24 julio	25 julio	1 agosto	24,46°C	24,18°C
18 octubre	17 noviembre	18 noviembre	9 diciembre	15,54°C	10,90°C

La temperatura media del período correspondiente corresponde a la suma de las temperaturas medias diarias dividido por el número de días.

El estudio de la correlación entre el número de días de desarrollo de cada fase y la temperatura media del período correspondiente da los siguientes resultados.

RESULTADOS

1) La duración (días) del desarrollo de la fase huevo-larva madura es directamente proporcional a la temperatura media del período (Fig.1) y obedece a la fórmula:

$$t = 61{,}295 - 2{,}089\ T$$

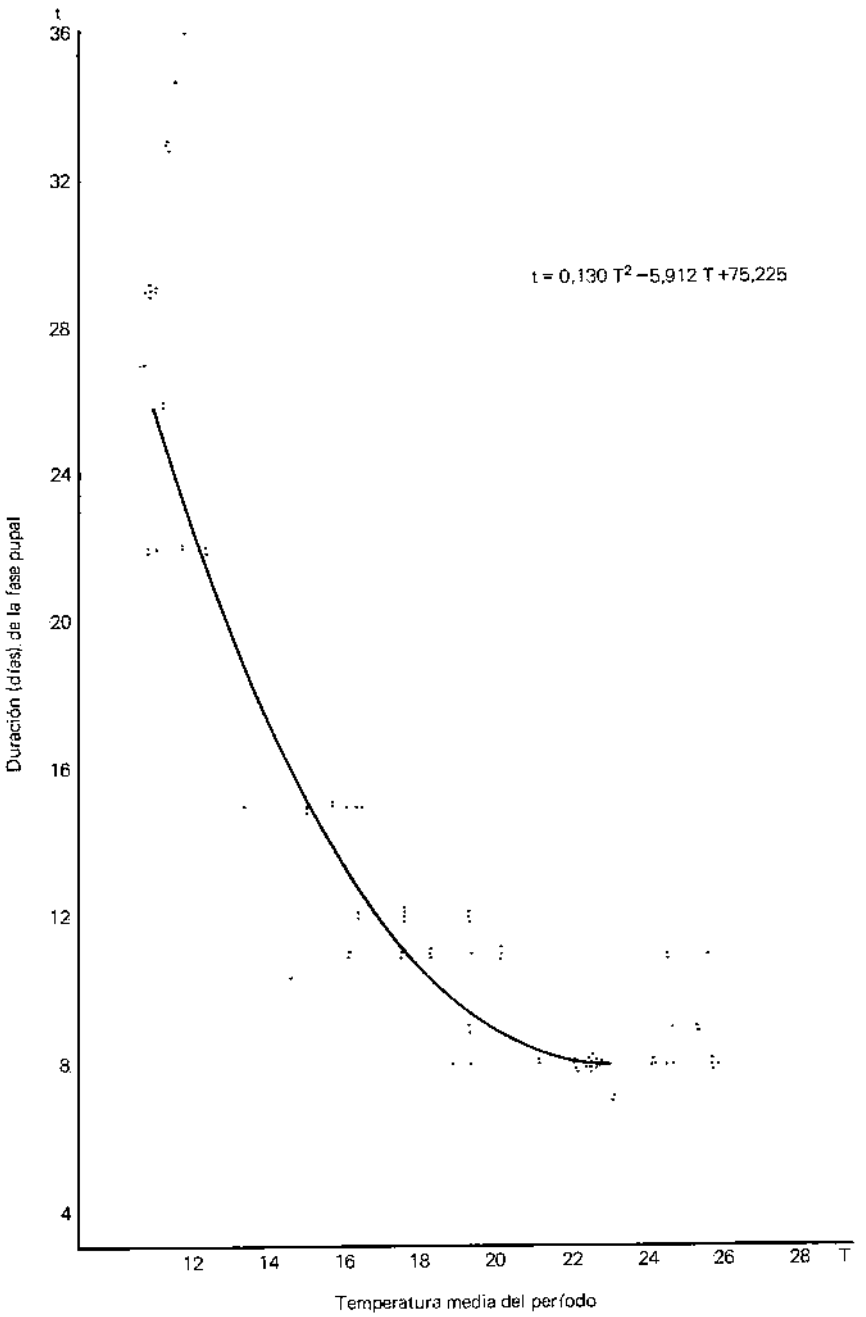

FIG.2. Período mínimo de la fase pupal (pupa-adulto) de C. capitata *Wied. en naranjas.*

De donde:
t = duración del período huevo-larva madura
T = temperatura media del período

Con una $T < 13°C$, la variabilidad es muy alta; con una $T = 24°C$, el período se hace constante (13 días).

	Análisis de varianza	
	G.1 (DF)	CM (MS)
Regresión	1	3707.987[a]
Resíduo	84	3.216
	$R^2 = 0,9321$	

2) La duración (días) del desarrollo de la fase pupa-adulto (Fig.2) obedece a la fórmula:

$t = 0,13\ T^2 - 5,912\ T + 75,225$

De donde:
t = duración del período pupa-adulto
T = temperatura media del período

Con una $T < 13°C$, la variabilidad es alta; con una $T = 22°C$ el período se hace constante (8 días).

	Análisis de varianza	
	G.1 (DF)	CM (MS)
Regresión	2	1597.653[a]
Resíduo	68	2.921
	$R^2 = 0,9415$	

CONCLUSIONES

A la vista de los resultados podemos predecir para la zona de la experiencia las sucesivas generaciones de *C. capitata*, a fin de tomar las oportunas medidas o bien después de un tratamiento insecticida con las consiguientes correcciones,

[a] Significativa al 0,001.

cuándo se producirán las máximas de población y, por tanto, poder anticipar el siguiente tratamiento insecticida.

En el caso de actuar con el método de los insectos estériles (SIT), se podrían liberar los insectos en los momentos más idóneos antes de alcanzar la población natural la madurez sexual.

DIFFERENTIAL STERILITY INDUCED BY GAMMA RADIATION IN THE ADULT MALES OF SIX STRAINS OF *Ephestia cautella*

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Throughout Iraq *Ephestia cautella* populations exhibit conspicuous genetic variations in the adult forewing pigmentation [1]. No more than three pure strains could be easily isolated at any time from the field within one or two generations [2, 3]. In the field there are no intrinsic mating barriers between these strains and their heterozygotes. The details of these strains have been fully described elsewhere [2].

It is interesting to note that these strains and one of their heterozygotes showed somewhat different tolerance to high or low temperatures suggesting that each particular genotype possesses different adaptive values [4]. Furthermore, it was also suggested that strains collected from different countries might respond differently to gamma radiation [5].

As Iraq is steadily moving towards using ionizing radiations for genetic or non-genetic methods of control, we decided to measure the response of 6–24-h-old adult males of different *Ephestia cautella* strains to 5 doses (10, 20, 30, 40, 50 krad respectively) of gamma radiation.[1] After treatment each male was mated to a virgin female. Fecundity, egg hatchability and mating frequency were measured. Table I shows the results of percentages of hatched eggs only in the tested strains. In this respect, significant differences in radiosensitivity were revealed

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[1] 1 rad = 1.00×10^{-2} Gy.

TABLE I. THE EFFECT OF 5 DOSES OF GAMMA RADIATION ON INDUCING STERILITY IN 6–24-h-OLD ADULT MALES OF 6 DIFFERENT STRAINS OF *Ephestia cautella* RELATIVE TO THEIR CONTROLS

Dose (krad)	Percentage egg hatch					
	Strains					
	C[a]	B[a]	A[a]	al[c]	D[a]	*we*[b]
10	61.6 (11)[d]	77.7 (14)	74.4 (6)	81.5 (11)	88.7 (6)	93.4 (18)
20	22.2 (11)	35.2 (14)	41.9 (7)	43.8 (9)	65.0 (9)	72.2 (20)
30	6.3 (15)	9.2 (12)	13.8 (7)	21.6 (9)	31.6 (9)	41.2 (17)
40	1.7 (13)	3.4 (17)	6.0 (7)	6.1 (9)	16.8 (7)	22.6 (20)
50	0.5 (15)	0.4 (15)	0.6 (6)	0.4 (9)	1.7 (8)	12.9 (16)

[a] Ref.[2].
[b] White eye [6].
[c] Albino, obtained from Stored-Product Insects Research & Development Laboratory, U.S.D.A., Savannah, Georgia, USA.
[d] Figures in parentheses represent number of pairs (replicates).

in some of these strains by applying pertinent statistical analyses. Thus, the C strain proved to be highly radiosensitive in comparison with, for example, the D or *we* strains. Other differences are mentioned in detail in the full text.

As previously stated [2, 3], the strains that have been isolated in Iraq according to the easily distinguishable forewing coloration, exist in nature at certain percentages side by side with their heterozygotes. Therefore it is thought necessary to take the significant differences in their response to gamma radiation into consideration if this radiation is to be used for inducing sterility to control this highly damaging insect or merely for dry date disinfestation purposes.

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PERFORMANCE OF TSETSE POPULATIONS FED ON RADIOSTERILIZED BLOOD CONTAINING GLUCOSE AND ATP

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Earlier investigations have proved that

(a) a 100-krad gamma irradiation of defibrinated pig's blood effectively inactivates bacteria strains which are frequently in the blood [1].[1]

(b) in spite of significant deterioration in important blood parameters [2], this sterilized blood is suitable for a long-term in vitro feeding of *Glossina morsitans morsitans* Westwood [3].

Experiments on the long-term feeding of irradiated blood containing glucose and/or ATP to *Glossina morsitans morsitans* Westwood and *Glossina palpalis gambiensis* are reported. Glucose can diminish the radiation damage in the erythrocytes [4]. ATP stimulates the blood intake by the insects [5]. Both substances improve the energy turnover and therefore, presumably, the nutritive utilization of the blood. Glucose was added to the diet before irradiation. The blood was stored up to 14 days at 4°C. ATP was dissolved in the blood immediately before feeding. The concentrations of both additives in the blood were 1 mg/ml. The conditions of the insect populations were characterized by the usual parameters: percentage of adults emerging from puparia; number of females; number of pupae produced per female; percentage of dead females per day; average pupal weight per month. Results are presented in Figs 1 and 2 for *G. morsitans* and *G. palpalis* respectively. In all these parameters the superiority

[1] 1 rad = 1.00×10^{-2} Gy.

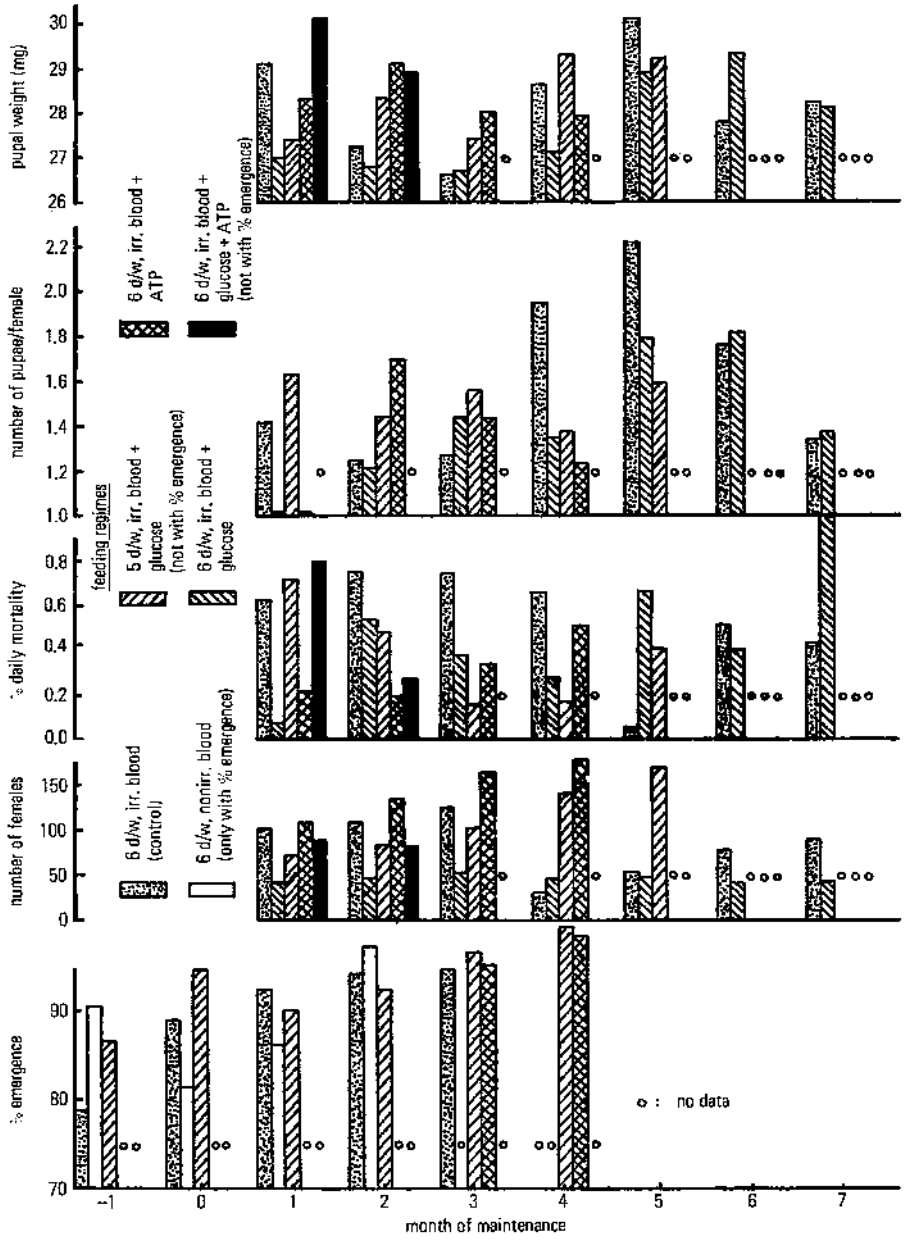

FIG.1. Monthly records of Glossina morsitans morsitans *Westwood fed on irradiated pig's blood containing glucose and/or ATP.*

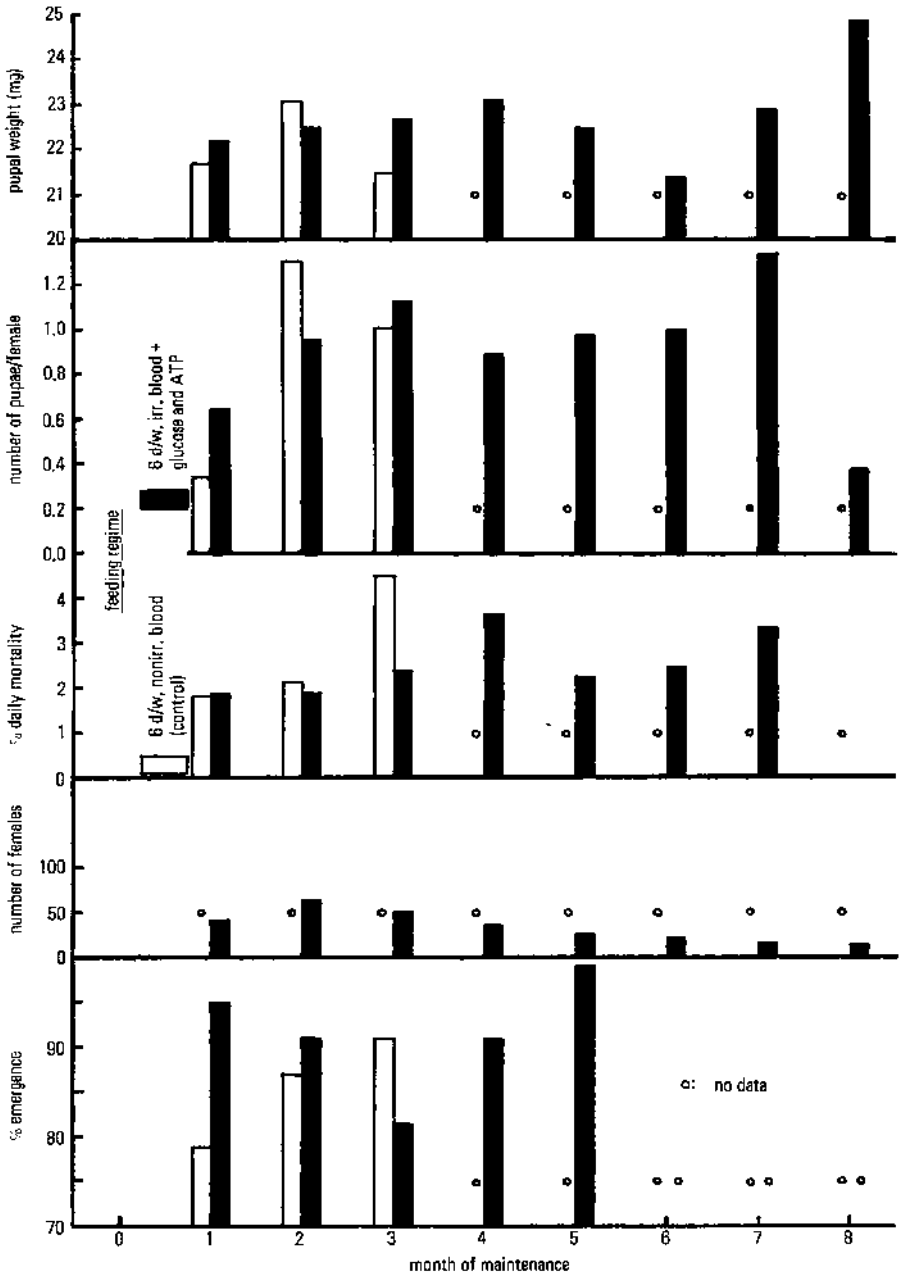

FIG. 2. Monthly records of Glossina palpalis gambiensis *fed on irradiated pig's blood containing glucose and ATP.*

of the flies that had received glucose, or (more significantly) ATP, was obvious after two months. Later the differences between the parameters for the various diets faded. If the flies were fed only on 5 days instead of on 6 days per week, the mortality of the insects was unchanged, but the number of pupae per female and the average pupal weight were significantly less for 2 and 4 months respectively. Incubation of the glucose containing blood at 37°C for 24 hours after irradiation did not improve the performance of populations.

We conclude from these observations that glucose, and especially ATP, influence the utilization of the irradiated blood in the in vitro feeding of tsetse positively, and affect favourably, although not greatly, the overall condition and development of a population.

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SUSCEPTIBILITY OF *Glossina palpalis palpalis* AT DIFFERENT AGES TO INFECTION WITH *Trypanosoma congolense**

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It has been emphasized repeatedly that SIT programmes must take into consideration that releases of large numbers of tsetse flies have attendant risks associated with transmission of disease [1].

With the aim of determining the vectorial capacity of gamma-irradiated males, reliable information on the infection rates of normal untreated flies has first to be obtained.

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TABLE I. AGE OF FLY AT THE TIME OF THE INFECTED FEED[a]

	Age of the males at the time of the infected feed															
	KADUNA strain		ZAIRE strain													
	DAY 1 (32 h)		DAY 1 (32 h)		DAY 2 (56 h)		DAY 3 (80 h)		DAY 3F (80 h)		DAY 5		DAY 10		DAY 15		DAY 25	
Group No. of flies	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.
1	14	1	29	8	5	0	5	0	5	0	5	0	5	0	5	0	3	0
2	14	1	28	3	5	0	4	0	5	0	5	0	5	0	4	0	3	0
3	14	1	26	2	5	0	5	0	5	0	5	0	5	0	3	0	4	0
4	15	3	28	3	5	0	3	0	4	0	5	0	5	0	5	0	2	0
5	15	2	30	1	3	0	3	0	5	0	5	0	4	0	5	0	5	0
6	14	1	28	5	5	0	5	0	4	0	5	0	5	0	4	0	3	0
7	15	2	29	8	4	0	4	0	4	0	5	0	5	0	5	0	5	0
8	15	1	28	3	5	0	3	0	5	0	5	0	4	0	5	0	4	0
9	15	3	29	6	5	0	5	0	5	0	4	0	5	0	5	0	5	0
10	15	2	30	3	5	0	5	0	5	0	4	0	4	0	5	0	5	0
Totals	146	17	285	42	47	0	42	0	47	0	48	0	47	0	46	0	39	0
Infection rates	11.6		14.7		0		0		0		0		0		0		0	

	Age of the females at the time of the infected feed															
	KADUNA strain		ZAIRE strain													
	DAY 1 (32 h)		DAY 1 (32 h)		DAY 2 (56 h)		DAY 3 (80 h)		DAY 3F (80 h)		DAY 5		DAY 10		DAY 15		DAY 25	
Group No. of flies	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.	No.	No. pos.
1	9	2	29	1	5	0	5	0	5	0	5	0	4	0	5	0	5	0
2	15	1	27	3	5	0	5	0	5	0	4	0	5	0	5	0	4	0
3	15	2	27	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0
4	13	1	28	4	5	0	5	0	5	0	5	0	5	0	5	0	3	0
5	15	1	29	1	4	0	4	0	5	0	5	0	4	0	4	0	4	0
6	13	1	27	1	5	0	5	0	5	0	4	0	5	0	5	0	5	0
7	14	3	30	4	5	0	5	0	5	0	5	0	5	0	5	0	4	0
8	15	2	30	6	5	0	5	0	5	0	5	0	5	0	5	0	5	0
9	15	0	26	1	5	0	4	0	5	0	5	0	4	0	4	0	4	0
10	15	1	27	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
Totals	139	14	280	23	49	0	48	0	50	0	48	0	47	0	48	0	44	0
Infection rates	10.1		8.2		0		0		0		0		0		0		0	

[a] Day 1, Day 2 and Day 3 flies were fed on an infected guinea pig within respectively 32 h, 56 h and 80 h of emergence. Day 3F flies, having taken a preliminary bloodmeal from a clean guinea pig, were infected between 56 h and 80 h after emergence.

The optimal age of the untreated tsetse fly for infection with trypanosomes of the brucei group has been studied intensively by several workers [2–5]. However, evidence on infection with *Trypanosoma congolense* is scanty [6, 7].

This study determines the relation of sex and age of untreated *Glossina palpalis palpalis* (Zaire and Nigeria origin) to infection by means of infective feed.

Stabilates of *Trypanosoma congolense* (TORORO/69/EATRO 1157) were injected into guinea pigs. Nine fly units of different ages (totalling 80 males and 80 females) were simultaneously fed on the same infected guinea pig at peak parasitaemia (only flies showing a red abdomen were selected). This procedure was replicated ten times, an infected bloodmeal using four guinea pigs being offered to 800 male and 800 female flies.

With the exception of the single infected feed, all flies were fed on clean guinea pigs. Before re-using the animals were examined for trypanosomes, cyclical transmission being found in one guinea pig.

On Day 30 post infection all the remaining live flies were dissected (Table I).

Infection was found only in flies offered an infected feed within 32 h of emergence. An overall infection rate of about 10% was found in these Day 1 flies. All other flies, 32-h-old upwards to 25 days at the time of the infected feed, remained negative.

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GENETIC APPROACHES; MASS REARING

Session 8

TEN YEARS OF TRANSLOCATIONS IN THE ONION FLY, *Delia (= Hylemya) antiqua*

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Abstract

TEN YEARS OF TRANSLOCATIONS IN THE ONION FLY, *Delia (= Hylemya) antiqua.*

Over a period of ten years more than 70 chromosomal rearrangements have been isolated for genetic insect control purposes. No significant difference was observed between X-rays and fast neutrons in the efficiency of translocation induction. Low doses around 2 Gy, mainly on 7-day-old males, were applied. On the basis of F_1-semi-sterility and cytological analysis, translocations and inversions were scored. Mostly only asymmetrical translocations were kept for further inbreeding. From 30 of such stocks finally two gave homozygous translocation lines. Re-irradiation of a Y-linked stock with normal fertility was carried out to induce complex Y-linked translocations for the development of a genetic sexing mechanism. Several combinations were observed, except the Y-2-4 translocation in which the dieldrin locus should be present. Following re-irradiation of viable homozygous translocations, in later studies multiple translocations were induced and some again were made homozygous. Further basic cytogenetic studies provided more insight into the behaviour of the chromosomes, which was used in these studies of structural rearrangements.

INTRODUCTION

In western Europe the onion fly is the only important insect pest of the onion, and in the absence of control measures larvae of this fly can cause much damage and therefore insecticides are generally applied. Until 1965 organochlorine compounds (DDT, aldrin, dieldrin and heptachlor) were mainly used, but subsequently these were replaced by organophosphorous compounds or carbamates. In the past ten years mostly the O-P-compound trichloronate has been exclusively applied to control the onion maggots. Following detailed studies on different aspects of the Sterile Insect Release Method (SIRM), another group at the Institute of Phytopathological Research has succeeded in applying this method to the onion fly. In a 40-ha large-scale field experiment it was demonstrated that effective control of the onion fly with SIRM is possible.
Ten years ago, at the Institute for Atomic Sciences in Agriculture (ITAL) and at the Department of Genetics of the Agricultural University at Wageningen,

studies were initiated to establish the feasibility of using structural chromosome mutations for genetic control of the onion fly. Mainly reciprocal translocations were investigated. Because of the absence of morphological markers the pseudo-linkage technique could not be used. By combination of inherited reduced egg hatch and cytological analysis of suspected families with semi-sterility, translocations were selected.

Translocations can be exploited in several ways for the control of insect pests. For the onion fly initially translocations were studied mainly as a way to reduce the population fertility and therefore their number. To reach this, inbreeding experiments are necessary for the isolation of homozygous translocation lines. More recently other approaches with the aid of translocations were considered, i.e. population replacement using insecticide susceptible alleles or the development of a genetic sexing mechanism.

INDUCTION OF RECIPROCAL TRANSLOCATIONS WITH RADIATION

Irradiation levels were established which produced a reasonable output of semi-sterile lines with a minimum of radiation damage caused by breakpoint damage and recessive lethals. This prerequisite of a low initial dose is very important for the chance to isolate homozygous translocations. Consequently, the accompanying disadvantage is that the initial screening to isolate translocations has to be on a larger scale. Although the early spermatid stage, based on *Drosophila* studies, is the most sensitive stage for the induction of translocations, in the onion fly it is impossible to synchronize spermatogenesis in such a way.

Mainly 7-day-old males (all sperm) were exposed to radiation, as this method is more efficient than the irradiation of fertilized females for translocation induction. Comparison between the translocation induction efficiency of X-rays and fast neutrons showed a non-significant difference. With a 2-Gy (= 200 rad) dose of X-rays or fast neutrons, an average of 8.5% and 7.5% of translocations respectively were recovered in the F_1 progeny. This may seem rather surprising as for dominat lethal induction in the onion fly fast neutrons are three times more effective than X-rays. It remains unclear why the difference in the translocation frequency between the two types of irradiation was not greater. However, with fast nuetrons many multiple translocations were induced, including a quadruple involving 4 out of 5 autosomes.

ISOLATION OF RECIPROCAL TRANSLOCATIONS

A method was developed to discriminate between eggs that hatched normally, eggs that did not hatch mainly because of non-fertilization and brown eggs caused

by unbalanced gametes. The presence of brown eggs, i.e. late embryonic lethals, is a good indication of the presence of a translocation. Detailed cytogenetical analysis of young embryos was carried out, and a clear relationship was established between the percentage of the two types of 'duplication-deficiency' embryos, descending from adjacent-1 configurations in M_I, and the percentage of brown eggs. Increased sterility in TN × TN crosses is not always a distinct indication for the karyotypical constitution of the parents. Therefore additional cytological analysis of the progeny of the suspected crosses gives the best success.

Mainly asymmetrical (exchanged segments unequal in length) translocations were used for inbreeding with the aim of isolating homozygous translocations. Next to meiotic pairing, somatic cross-configurations in larval brain mitosis enabled scoring at an early stage of the rearing. However, for the isolation of homozygotes, cytological analysis of adult males rather than larvae gives the most reliable results.

Meiotic orientation of translocation multiples was studied in several translocations (A-A, Y-A, X-A and Y-A-A). Because of the absence of chiasmata in the males translocation complexes usually behave and disjoin differently in males and females. Adjacent-II and numerical non-disjunction orientations were mainly observed in females of an autosome-autosome and an X-linked translocation respectively.

Elementary cytogenetic studies provided more insight into chromosome behaviour and sex determination. Different types of X- and Y-chromosomes are present in natural populations, and sex-ratio distortion could be explained by these phenomena.

Crosses between X-linked translocation males and particular inversion females delivered compound chromosomes in the larval progeny. Further translocations were used as a translocation tester set to locate the Adh gene on chromosome 6.

Over 70 chromosomal rearrangements were isolated of which 42 were asymmetrical reciprocal translocations and 17 symmetrical. Among the asymmetrical category, seven had 'duplication' larvae and 35 had none.'Duplication' larvae are descended from a particular gametic combination of a normal and an adjacent-1 gamete which carries a large duplication (cytologically recognizable) and a short deficiency. Such larvae can almost reach the pupal stage and in some translocation stocks they are even viable as adults (but not fertile). A significant difference in fertility between translocations with and without 'duplication' larvae was established, through the survival of the 'duplication' eggs beyond the hatching phase. Nine triple (six cyclic and three non-cyclic) translocations and one quadruple were observed. With increasing complexity the semi-sterility increases as well. Two pericentric inversions and three combinations between a translocation and an inversion were observed. Absence of sterility in inversion-heterozygous males confirmed the absence of crossing-over in this sex.

Calculation of the number of translocation breakpoints in relation to chromosomal length indicated that relatively more breakpoints were present in chromo-

somes 2 and 6. These data are important for further genetic control studies, as will be shown below. When a particular beneficial gene, e.g. dieldrin resistance, is located on a chromosome which is not often involved in a translocation, further progress in the development of a genetic sexing mechanism will be retarded.

ISOLATION OF TRANSLOCATION HOMOZYGOTES

Over 30 stocks carrying translocated segments of a reasonable length and with a reasonable difference in size were inbred. About half of them showed homozygosity in the larval stage. Five of these homozygotes reached the adult stage and two stocks could finally be reared as a stock. One of these, an X-linked translocation, was less fit compared with the control, but the other autosome-autosome translocation was as fit. This translocation homozygous stock was released into a field cage, together with a standard laboratory strain. During the course of the season the fecundity and fertility of the adults were measured together with the karyotype frequencies of the F_1 progeny. No selective disadvantage in the translocation karyotypes was observed.

Because of diapause response of the stocks (most pupae entered diapause), the effect of the reduced fertility could not be followed in this field-cage population. This phenomenon emphasizes the importance of the quality of the laboratory stocks to be released. After re-irradiation of this autosome-autosome translocation, eight three-choromosome double-translocation heterozygotes were isolated. Three of these were viable as adult homozygotes.

It was concluded that even with these multiple translocations a stable equilibrium is difficult to reach. Firstly, the frequencies of duplication/deficiency gametes are too low; secondly, there is insufficient complementation; and, thirdly, there is too much recombination in the differential segments, resulting in undesirable karyotypes.

GENETIC SEXING

Following re-irradiation of a Y-linked translocation stock (Y-2) with normal fertility, several complex Y-linked translocations were induced. Unexpectedly, the Y-linked translocation had normal fertility because of a complete alternate co-orientation of the translocation complex. Only chain quadrivalents were observed, and therefore probably adjacent-1 is unstable and will not occur. From 112 semi-sterile progenies, 62 had translocations of which 13 (18%) were complex Y-linked translocations.

This percentage was in agreement with the expectation based on the total length of chromosome Y and 2 (21%) of the initial translocation. Different

combinations between Y-2 and the other autosomes were established cytologically. Unfortunately no complexes with chromosome 4 were isolated, whereas crosses of heterozygous dieldrin-resistant F_1 males (from irradiated fathers) with susceptible females gave no indication of linkage with one of the other autosomes.

Studies on the development of a genetic sexing mechanism based on ethanol and Adh were discussed after our successful approach in *Drosophila*, but the absence of an Adh-null allele is a major obstacle for this approach.

CONCLUSION

Many genetical, cytogenetical and biological data have been obtained over the past ten years. In spite of the collection of these data some genetic studies are still outstanding. For example, how can we improve the isolation of suitable homozygous translocations, how can we obtain translocations with higher sterilities, how can we solve the diapause problem in laboratory stocks? It is still not known why no morphological or visible mutants could be observed. Such mutants would enlarge the screening efficiency using the pseudo-linkage technique. Further experiments on a field scale are not planned, although the development of a genetic sexing system for the ongoing sterile male project would be attractive.

A list of the relevant literature on onion fly genetics, cytogenetics and population dynamics can be supplied by the authors.

GENETIC MARKERS

Discovery and use in insect population dynamics studies and control programmes

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Abstract

GENETIC MARKERS: DISCOVERY AND USE IN INSECT POPULATION DYNAMICS STUDIES AND CONTROL PROGRAMMES.

A successful marking technique for insects used in sterile insect control programmes or in population dynamics studies should have: (1) ease of application, (2) minimal manipulation of insects, (3) ease of recognition, (4) certainty of persistence through life stages and (5) relative freedom from deleterious biological effects. Mutations, whether morphological, biochemical or chromosomal fit these criteria. The discovery of useful genetic markers is usually a simple matter of examination of existing laboratory stocks, although ionizing radiation or mutagenic chemicals can also be used to induce simple mutations. The various inheritance patterns that can be expected for a genetic marker are: (a) recessive, (b) co-dominant, (c) dominant, (d) sex-linked (and e) polygenic. The usefulness of mutations as markers usually fits the following pattern: co-dominant > dominant > recessive > polygenic; although the existence of a recessive simply inherited marker can be extremely useful in studies that involve only one generation. Body and eye colour mutations are the most common and most conspicuous markers found in insect species. Isozyme markers are normally present in insects, but require chemical techniques for visualization. Chromosomal polymorphisms also require rather sophisticated techniques and knowledge for accurate interpretation. Thus, for ease of recovery and identification, morphological mutations offer good tools for studying insect populations in the field. Genetic markers have been used successfully in the cabbage looper, the boll weevil and the pink bollworm to investigate population dynamics in field populations. In the boll weevil the co-dominant mutation, ebony, has been shown to be equal to wild-type strains in viability and persistence in populations. Failures in sterility induction (or recovery of fertility in the released insects) in a boll weevil SIT programme can be detected by the presence of hybrid ebony weevils in the release zone. The dominant body colour mutation, sooty, in the pink bollworm has been used to follow population trends in field populations, and to show direction and distance of dispersal of released insects.

1. INTRODUCTION

As entomologists have developed and used the Sterile Insect Technique (SIT) over the past 25 years, two aspects of the system have consistently been found deficient. The first is the paucity of information on the natural reproductive behaviour of most pest species [1], and the second is the lack of quantitative methods of pre- and post-treatment population assessment [2]. Some of the best information on reproductive behaviour is obtained through direct observation of the insects in the field [3]. However, this method is cost and labour intensive and requires well-trained and dedicated observers (especially for nocturnal species). Assessment of insect numbers in natural populations has recently been aided by the use of pheromone trapping, nevertheless there are still some nagging questions related to the effectiveness of pheromone usage and interpretation [4]. Insect marking techniques [5] such as dyes, mutilation, paints and radioactive isotopes have long been used as tools to find answers to both problems (assessment of reproductive behaviour and population size), but often the marking technique is also costly and sometimes detrimental to the released insects. Five conditions of a successful marking technique for insects used in population dynamics studies or in sterile insect control programmes are: (1) ease of application of the marker, (2) minimal manipulation of insects to apply the marker, (3) ease of recognition of the marker by laboratory or field personnel, (4) certainty of persistence of the marker through life stages and (5) relative freedom from deleterious biological effects caused by the marker. Mutations, whether morphological, biochemical or chromosomal, fit these criteria as well as or better than physically applied marking techniques.

The use of biochemical genetic markers for the study of insect behaviour and ecology has been discussed recently by other workers [6, 7]. Isozyme variation is almost always found in insect populations, and such variation often appears to be unaffected by natural selection [8]. However, the electrophoretic technique used to visualize the electromorphs is costly, slow and difficult to use under field conditions. The use of visible genetic markers as aids to assessment of insect reproductive behaviour and population dynamics is discussed here. The combined use of direct field observation and visible genetic markers to study reproductive behaviour is possible in most economic species. Pre- and post-treatment population assessment using mutations in a release-recapture experiment can yield valuable information on migration, immigration and population density.

2. ISOLATION AND MAINTENANCE OF VISIBLE GENETIC MARKERS

Visible genetic markers have been reported from many laboratories which rear insect species. In fact, references to mutations in insects are too numerous

to list here. Two good reviews of genetic strains in important insect species are available [9, 10]. Often, unfortunately, if one tries to obtain cultures of such mutants from the reporting laboratory, the mutant strain has been discarded or lost. I believe this situation can be altered by two considerations. First, attention must be paid to certain husbandry techniques which will help maintain the strains. Second (and probably more important), investigators and administrators must recognise the value of such mutant strains in research programmes, so that the extra time and money needed to maintain separate strains will be made available. The first point will be examined in this section. The value and uses of mutant stocks of insects is the subject of Section 3 below.

2.1. Examination of laboratory cultures

Almost every economically important species of insect is in culture in some laboratory in the world. These laboratory cultures are the best source of mutant genes. Many times a simple but detailed examination of the individuals in the laboratory culture will reveal genetic variation existing in the population. All stages of the insect should be examined, since mutant genes can affect any stage of development. Table I lists possible morphological characters that can show genetic polymorphism in each stage of the insect's life. Each of these suggested variations has been found in insect species in the past. I will discuss the steps necessary to confirm the mutant character of a suspected aberration in Subsections 2.3. and 2.4 below.

If examination of the laboratory population does not reveal any morphological variation which may be inherited, the next step is to inbreed the population in order to make it possible for recessive mutations to be seen. The purpose of inbreeding is, of course, to increase homozygosity in the population. In general, inbreeding is regarded as detrimental to a population and so is studiously avoided, but to recover recessive mutations it is not only desirable but necessary. Once a mutation is isolated and purified (made homozygous) then steps can be taken to recover heterozygosity in the mutant population, if that is a desirable condition. A possible inbreeding scheme is shown in Fig.1. A number of single pair crosses are extracted from a random mating laboratory population. The number of single pair crosses carried at any time depends upon the available space and labour in the rearing facility. The probability is very high that 100 single pair matings will reveal a minimum of 1 simply inherited recessive morphological mutation. The most common mutants will be colour changes, usually eye- or body-colour mutations, which can be detected in the larval or adult stages. Recessive mutants will be present in the population mainly as heterozygotes, but will appear (phenotypically) normal. A homozygous mutant is shown in the laboratory population in Fig.1 for illustrative purposes in cross No.4. Unless the frequency of the mutant allele is fairly high (> 0.1),

TABLE I. CHARACTERS THAT MAY SHOW POLYMORPHISMS IN VARIOUS STAGES OF AN INSECT LIFE CYCLE

Body part	Genetic variation in			
	Egg	Larvae	Pupae	Adult
Integument	Colour Size Shape	Colour Size Pattern Protuberance (position or presence)	Colour Size Pattern Protuberance (position or presence)	Colour Size Pattern
Leg	–	Shape Colour	Shape Colour	Shape Colour
Wing	–	–	Shape Absence Colour Number	Shape Absence Colour Number
Eye	Colour	Colour Number	Colour Shape Size	Colour Shape Size
Scales	–	–	–	Colour Pattern Absence
Antennae	–	–	Absence Shape Size	Absence Shape Size
Testis	–	Colour Size Position	Colour Size Position	Colour Size Position
Bristle	–	–	–	Number Shape Pattern

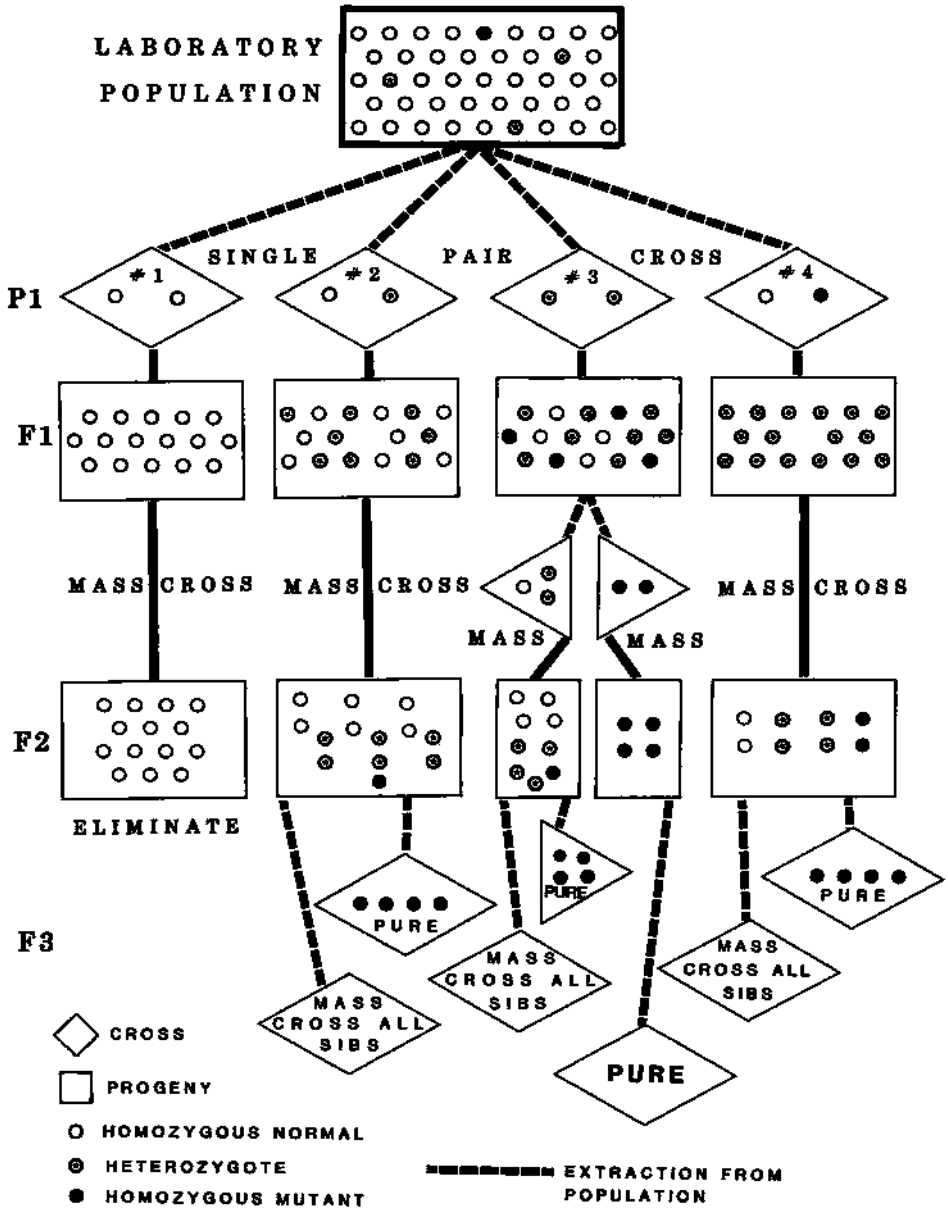

FIG.1. Schematic representation of an inbreeding system to recover existing or induced mutations from a laboratory population of insects. Crosses Nos 1 to 4 illustrate all possible mating combinations, but not all are equally likely to occur.

or the population size is very large, there may not be homozygous mutants present. Crosses Nos 1 to 4 illustrate the possible matings that could occur in the single pair crosses. Cross No.1 will be the most frequent type of cross. Cross No.2 will be most likely to yield mutant progeny in the F_3 generation. Note that the phenotypes of individuals in crosses Nos 1, 2 and 3 are all alike, since the heterozygotes are not phenotypically recognizable with a recessive mutation. If, by chance, two heterozygotes were crossed as in No.3, then a pure strain could be established in one generation.

If the hypothetical mutation illlustrated in Fig. 1 were dominant, or co-dominant, then crosses Nos 2, 3 or 4 would be possible, depending on gene frequency. In this case pure cultures could quickly be obtained.

2.2. Treatment by mutagenic agents

In the unlikely event that mutant individuals are not recovered by examination of existing laboratory cultures, then insects can be treated with ionizing radiation or chemicals to induce mutations. In fact, it is possible to combine such treatment with the inbreeding scheme shown in Fig.1. If spontaneous recessive mutations exist in the laboratory population, then inbreeding will uncover those mutants at the same time that newly induced mutants are being isolated (as in cross No.2, Fig.1). The one disadvantage of combining these steps is the fact that recessive detrimental mutations may be induced by the treatment and lower the fitness of the visible mutation strain. Rigorous inbreeding combined with artificial selection for vigour and reproductive performance will generally eliminate such detrimental mutations in two or three generations.

It is not possible here to specify the exact conditions of irradiation or chemical treatment needed to maximize recovery of visible mutations. The radiosensitivity of the species and conditions of treatment will cause such predictions to lack much validity. However, enough data is available from *Drosophila* [11] to allow some rough estimates of mutant yield and to specify some general treatment levels. The mutation rate for visible autosomal loci ranges from around 1×10^{-4}/R per locus to 1×10^{-8}/R per locus. Examination of lists of mutants of several species of insects [9] shows that the numbers of known loci range from about 40 in *Tribolium* to thousands in *Drosophila*. I believe that on the average one could readily expect to find 100 recognizable characters that could change in an insect. The choice of 100 not only simplifies the arithmetic that is to follow, but is also reasonable based on numbers of existing mutant stocks in *Bombyx*, *Tribolium* and *Aedes aegypti* [9]. Assuming a linear relationship between radiation dose and frequency of induced mutations (in the range of doses to be considered here), we may express the number of

TABLE II. NUMBER OF RECESSIVE AUTOSOMAL MUTATIONS EXPECTED FOR 2 EXTREME MUTATION RATES AND 5 DOSES OF RADIATION, ASSUMING 100 LOCI PER INDIVIDUAL CAN MUTATE TO A USEFUL VISIBLE MORPHOLOGICAL CHARACTER

Dose (R)	Number of mutations per individual at	
	1×10^{-4} Mutants/R	1×10^{-8} Mutants/R
500	5	0.0005
1000	10	0.001
5000	50	0.005
10000	100	0.01
50000	500	0.05

mutations (N) expected per individual from a given dose of radiation (D) to be as follows:

$$N = \mu LD$$

where μ = mutation rate and L = number of loci expected to mutate to a usable allele. High and low estimates of N are given in Table II for various doses of radiation. The dose of 500 R is probably too low to yield any mutations in the number of F_1 individuals which could be comfortably handled. But by using 1000 R, even at the lowest estimated mutation rate an investigator could expect, on the average, one usable visible autosomal mutation from 1000 F_1 progeny. This estimate does not take into account sex-linked mutations, nor dominant mutations. Thus, if induced mutation rates for these other classes of mutants are similar to those for autosomal recessive mutations, three times as many mutants as shown in Table II could be obtained in a particular test. If both parents are irradiated then the expected number of mutants could be doubled.

2.3. Detection of mutations

When an aberrant individual is found in a colony of insects we need to determine if the aberration is inherited and how it is inherited. If a mutant allele only shows its phenotypic expression when two identical alleles are present in the genotype of the insect (i.e. when it is homozygous), then the allele is said to be inherited as a recessive mutation. If only one of the mutant alleles need be present in the genotype for full expression of the phenotype, the allele is

said to be inherited as a dominant mutation. If an insect shows one phenotype when it is heterozygous (i.e. it has only one of the mutant alleles in the genotype) but a different phenotype when it is homozygous, then the mutant is inherited as a co-dominant mutation (there are several terms used to designate this type of inheritance depending upon the strength of appearance of the mutant in the heterozygous genotype; I shall use co-dominant here to indicate that the heterozygote is distinguishable from either homozygote).

Dominant and co-dominant visible mutations are the easiest classes of mutants to detect, but the least frequent in occurrence. Such mutations will be seen in the first generation after treatment and will occur in 50% of the progeny of an affected individual (because they are usually found as heterozygotes). Dominant mutations are valuable in field release programmes since not only the homozygous released individuals are marked but any progeny of matings with the released individuals will also be marked. Co-dominant mutations are even more valuable in this regard because the released homozygotes can be distinguished from F_1 progeny of matings between the released insects and native insects. Many mutants in this class turn out to be homozygous lethals, and may have value as both markers and as control tools [12]. Many melanic body colour mutations in insects are inherited as dominant or co-dominant mutations [10, 13, 14]. Individual insects that do not express the mutant trait will not pass the trait on to their progeny. I will not consider here the phenomena of variable expressivity and reduced penetrance which may occur with any trait, but generally reduces the value of the mutation as a marker if either condition prevails.

Recessive visible mutations will only be seen when two parents heterozygous for the mutant allele are mated and homozygous progeny are produced. Thus, we can expect recessive characters to appear after two generations of inbreeding, as shown in Fig.1 (cross No.2). Eye-colour mutations are often inherited as recessive alleles [13, 15]. Changes in patterns of scales in lepidoptera (colour patterns) are also often recessive [10]. Recessive mutants can be used as markers in release programmes if only one generation of observation is of interest. When a homozygous recessive individual mates with a native insect, all the progeny will be wild-type (i.e. resemble the native parent). This is generally true because particular mutant alleles are usually rare in native populations. However, in some cases recessive mutants have been found in significant frequencies in populations [10, 16]. Once a recessive mutant stock is established, it is wise to collect native insects from the area of presumptive release and mate these to the pure marker stock to see if the mutant allele exists in that population [16].

A third class of mutations, called sex-linked visible mutants [14], can also be found. Sex-linked mutations occur on the chromosomes which differ in males and females. In most insects females have 2 X-chromosomes and males have 1 X- and 1 Y-chromosome. In lepidoptera and occasionally in

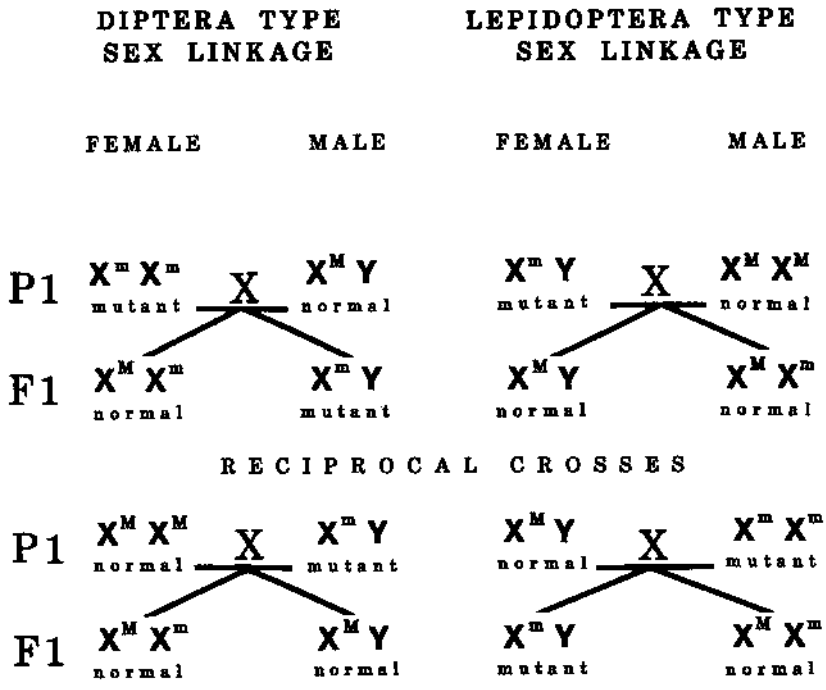

FIG.2. Types of progeny (F_1) resulting from reciprocal crosses of individuals (P_1) carrying a sex-linked mutation. Illustrated on the left are progeny distributions when the male is the heterogametic sex (diptera, coleoptera, etc), and the right side illustrates the case where the female is the heterogametic sex. In each case the Y-chromosome may or may not be present, depending on the species.

other species of other orders, this scheme is reversed (males are XX and females are XY). The Y may or may not be present. If a mutation occurs on the X-chromosome, then reciprocal crosses of the mutant with wild-type will give different results. The two types of sex-linkage are shown in Fig.2. Recessive sex-linked mutations are easily detected in the sex which contains heterologous chromosomes since only one representative of a locus is present. Thus, sex-linked mutants can be seen in males of diptera, coleoptera, etc. (or in females of lepidopteran species) in the first generation after treatment. Sex-linked characters are useful in field release programmes if one is interested in sex-ratios in the native strain. Also, such characters can be followed through two generations, even if recessive, if the homogametic sex is released carrying the marker and the opposite sex is sampled in the next generation. An example of this method in lepidoptera would be the release of genetically marked females (homozygous for a sex-linked marker) and recovery by F_1 males in pheromone traps.

A number of traits in insects are controlled by more than one locus. Such traits are called polygenic traits. In general, polygenic traits are not useful as genetic markers, since they are not usually easily seen. Reproductive characters, body weight, body size, insecticide resistance, diapause ability, and bristle number are traits that can be controlled by polygenes. This is not to say that such characters cannot be simply inherited. Populations of insects homozygous for a polygenic character can be released for population dynamics studies, if such populations exist and if the genetic characteristics of the strain have been determined.

The usefulness of mutations as markers in dynamics or control experiments usually fits the following pattern (where > means better than): co-dominant > dominant > sex-linked > recessive autosomal > polygenic. However, the usefulness of a mutant as a marker will depend a great deal on its competitiveness, viability and appearance (visibility) regardless of the inheritance pattern it shows.

2.4. A mutant recovery scheme

The following breeding system can be used to recover mutations in almost any insect species. The system assumes that the species can be laboratory reared, and that single pair crosses are successful. These are not absolute criteria, but the scheme must be adapted if these assumptions are not met. (See Fig. 1 (cross No.2) for a diagram of this scheme for autosomal genes only.) Sex-linkage in the following is assumed to be of the female = XY (lepidoptera) type; to convert to diptera-type sex-linkage, substitute male for female where underlining occurs. Try to ensure female virginity in crosses.

A. Irradiate (or chemically treat) both males and females (crosses can be in groups or single pairs).
B. In the F_1 progeny look at all male and female progeny for autosomal and sex-linked dominant or co-dominant, and sex-linked recessive mutations in female progeny.
C. Make single pair crosses of F_1 progeny. Set up as many single pairs as you can handle. Keep track of each family's (single pair) progeny.
D. Count numbers of F_2 males and females in each family; a deviation from normal sex-ratio will indicate presence of a sex-linked lethal. Examine females for sex-linked recessives induced in their grandfathers.
E. Mass cross F_2 families (brother X sister). Do not cross-mate families.
F. Examine all individuals in each family for recessive autosomal mutations. In the F_3 you will expect 1/16 of the progeny of a cross in which a recessive mutation was induced to be of the mutant type. Using Table II you can calculate the number of families expected to show a mutation, based on the dose used.

G. The frequency of mutants in confirmed F_3 families can be increased from 1/16 to 1/4 if single pair crosses are made in the F_2, rather than mass crosses. This increases the work significantly and it seems more profitable to handle more F_2 families, thus increasing the chance of finding a family containing a mutation.

H. When a mutation is found in a family at any of the above steps, two types of mass crosses are to be made: (1) cross all mutant individuals together, (2) cross all wild-type individuals together. Cross 1 will increase the mutant in pure culture, while cross 2 will produce a proportion of mutants in the next generation.

I. Continue to build up and to purify mutant stock. Recessive and co-dominant mutations are easily purified since only homozygotes are picked for mating, but completely dominant mutants are very hard to purify unless single pair crosses and progeny testing is carried out.

3. RELEASE-RECAPTURE EXPERIMENTS USING VISIBLE GENETIC MARKERS

A prime consideration for any insect marking technique is that the marker have a minimal effect on the behaviour of the marked individuals. Visible genetic markers can be found in most species that do not markedly affect field behaviour of laboratory-reared insects. In most population dynamics studies the marker will not be expected to last more than one or two generations, thus slight selective disadvantages should not be of great concern (assuming the investigator knows the extent of those disadvantages). Previous work using visible genetic markers has been criticized on the basis that visible genetic markers are rare in native populations because natural selection acts against the recurring mutant allele [8]. It is hard to see how such selection could seriously affect the results of a one or two generation release-recapture study. There is a possibility that laboratory domestication could affect the behaviour of released insects [17], but this possibility must be faced in any genetic control programme, such as SIT. It is my contention that adequate preliminary investigation of the colonized insect will often show how to compensate for any observed deficiencies in the released strain. In the remainder of this section, I will show how visible genetic markers in three economically important species were used to study population dynamics relative to genetic control programmes.

3.1. Dark cabbage loopers

A melanic mutation, dark, in the cabbage looper, *Trichoplusia ni* (Hubner), produces black body colour in the heterozygous state and acts as a recessive

lethal in the homozygous state. A field test of the ability of dark males to mate with native females was carried out on a 700-acre farm[1] in Maricopa County, Arizona [12]. The dark strain used in the test was placed in an outside insectary where temperature fluctuated with the ambient conditions to precondition the pupae to the field environment. Releases were made for approximately two months during the summer. During the period of the releases the cabbage looper populations were monitored by pheromone traps and larval collections in the field. Population densities were estimated from release-recapture data made on a weekly basis. The experiment showed conclusively that this dominant body colour mutation could be used to estimate population density in the field even though it is lethal in the homozygous state. In addition we recovered dark individuals (adults and larvae) from the field population three weeks after releases were terminated. These individuals could have only been F_1 progeny of the released males, thus demonstrating the ability of the released insects to interact with the native population.

3.2. Ebony boll weevils

A co-dominant melanic mutation, ebony, in the boll weevil has shown great promise as a marker for identification of released boll weevils [13]. The advantage of this genetic marker is that both the released individuals and the F_1 progeny of those individuals can be distinguished. The ebony mutation has been shown to be capable of persisting over nine generations in competition with its wild-type allele in both laboratory cage and field cage experiments [18]. In this same test heterozygous ebony boll weevils were found up to two miles away from the release point.[2] These heterozygotes were F_1 progeny of the released ebony adults and native insects, thus demonstrating that the released insects could mate with the native insects, and that insects carrying the mutant allele could be followed during dispersion. Since the original demonstration of the value of the ebony in boll weevil release-recapture experiments, the gene has been incorporated into the mass-reared strain used for sterile insect release programmes, and this genetically marked population has been used extensively for experimental purposes [19].

3.3. Sooty pink bollworms

A dominant melanic mutation, sooty, reported in the pink bollworm [14], has been subjected to tests of its fitness for use in a SIT programme. Larval viability estimates of individuals carrying the sooty allele ranged from 75–96%

[1] 1 acre = 0.405 hectare.

[2] 1 mile = 1.609 km.

of wild-type [14]. The rate of development of larval and pupal stages of the sooty strain was found to be significantly faster than a wild-type laboratory strain and the same as a native strain of pink bollworms [20]. Sooty was shown to have no effect on response to radiation as measured by induction of sterility in males when compared with males of other laboratory strains or with native males [21]. The sooty allele was demonstrated to persist over 11 generations in competition with a wild-type laboratory strain, and over a full cotton-growing season in competition with native insects [22]. Sooty individuals were capable of diapausing under field conditions and emerging in the spring during the same period that native insects were emerging. Released sooty males and their male F_1 progeny were attracted to and captured in pheromone traps. Sooty males were easily distinguished from wild-type pink bollworms in both live-male pheromone traps and in sticky traps in a large-scale field programme [23]. When sooty moths were introduced into a field population over a very short interval it was possible to observe population developmental rates in the field by using daily trap counts and boll collections to observe the first appearance of sooty progeny and to track the sooty population build-up over time [23]. When sooty individuals are present in low frequencies in native populations the allele appears to have a selective advantage [22].

4. SUMMARY AND CONCLUSIONS

Insects bearing artificially induced markers have been introduced into native populations of insects for the purpose of studying population dynamics and reproductive behaviour in many studies in the past. Often these markers are difficult to apply, affect the insect behaviour, have a high application cost and/or cannot be observed under field conditions. The use of visible genetic markers can help an investigator to avoid these problems in many species. Once a mutant strain is established, then it can be reared and released at the same cost as a normal laboratory strain of insects. Little training is required for workers to recognize the mutation in the field. The effect of a mutation on behaviour is of scientific interest as many studies in evolutionary phenomena have shown, but, as has been demonstrated in Section 3 above, the proper choice of mutations can avoid any behavioural problems that would affect the results of a particular experiment.

The recovery of valuable visible mutations in an insect species is usually a simple matter of systematic breeding and close observation of individual insects. Acquisition of good mutations is not nearly as difficult, in my opinion, as convincing investigators and administrators that such mutations are valuable in entomological research.

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ENZYME POLYMORPHISMS IN *Ceratitis capitata* Wied.

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Abstract

ENZYME POLYMORPHISMS IN *Ceratitis capitata* Wied.

Studies on enzyme polymorphisms have revealed much biological variability. Different enzyme forms generally can be recognized both in the homozygous and in the heterozygous conditions, so they are very suitable as markers in the analyses of genotypes as well as in the study of developmental differentiation. Some enzyme variants may cause physiological troubles, which could possibly be exploited for applied purposes. In work on electrophoretic enzyme polymorphism, electrophoresis and isoelectrofocusing are currently used. Zone electrophoresis is technically easier, and isoelectrofocusing has a much higher resolving power. In studies on the medfly the authors have concentrated on the enzymes that control glucose degradation and its various pathways. They have screened eight medfly strains for 12 enzyme systems; some of these systems are represented by two or more isozymes, so the different enzyme functions so far examined total 17. In all, this screening programme has covered 136 combinations, 34 of which have shown enzyme polymorphisms; 19 of these were due to electrophoretic mobility, five to enzyme activity, and 10 to both mobility and activity. The different forms of a given enzyme may show tissue and/or developmental stage specificity. All strains have shown at least one polymorphic enzyme system, with conspicuous differences between strains. The greatest variability amounts to nine polymorphic enzyme functions which have been observed on samples of the second laboratory generation of the Sardegna strain. The relative frequencies of the various enzyme forms generally do not differ between sexes. The only exception in the authors' records comes from a single observation on function PGM_1 in the Sardegna strain, based on 41 flies, and was associated with the absence of one homozygous class of flies. In other instances one of the two variants has not been found in the homozygous condition. By proper statistical treatment, the data already available should provide clues for the detection of genetic linkage between some of the variants so far studied.

1. INTRODUCTION

The expression 'enzyme polymorphism' refers to the different forms of activity which a single enzyme may display, either in a single individual or in different individuals of a genetic population. The different forms of activity of a single enzyme system may involve either properties directly related to enzyme function, such as activity level and protein stability, or properties related to

TABLE I. VARIABILITY OF 17 ENZYME FUNCTIONS OBSERVED IN EIGHT *Ceratitis capitata* LABORATORY STRAINS[a]

Enzymes	AK		HK				GOX	G6PD	6PGD	PGM		PGI	MPI	α-GPDH	HAD	FH	XDH
Isozymes	AK_1	AK_2	HK_1	HK_2	HK_3	HK_4	–	–	–	PGM_1	PGM_2	–	–	–	–	–	–
Italy	M	M_F	M_S	P*	M	M_S	M	P	M_F	M_F^*	M	M	P*	P	M	M	P
J.R.C. Ispra	M	M_F	M_S	P	M	M_S	M	M_S	M_F	M_F	M	M	P*	M_S	M	M	M_S
Sardegna	M	P	P	P*	M	P	M	P	P	P	M	M	P*	M_S	M	M	P
Costa Rica	M	P	M_S	P	M	M_S	M	M_S	M_F	M_F	M	M	P*	M_S	M	M	M_S
dark pupae	M	M_F	M_S^*	P	M	M_S	M	M_S	M_F	M_F	M	M	P*	M_S	M	M	M_S
white pupae	M	M_F	M_S	P	M	M_S	M	M_S	M_F	M_F	M	M	M_S	M_S	M	M	M_S
orange eye	M	M_F	M_S^*	P*	M	M_S	M	M_S	M_F	M_F	M	M	P*	P	M	M	P
apricot eye	M	P	M_S^*	P	M	M_S	M	M_S	M_F	M_F	M	M	P*	M_S	M	M	M_S

[a] M = Monomorphic (one mobility class)
M* = Activity and/or structural variability
M_S = Monomorphic slow
M_F = Monomorphic fast
P = Polymorphic (two mobility classes)
P* = Activity and/or structural variability in both mobility classes.

analytical techniques, such as electrophoretic mobility, or both. From a functional point of view activity and/or stability variants are of greater interest, whereas electrophoretic mobility is the tool of choice for the study of genetic variability.

Enzyme proteins are generally coded by single specific genes. Genes of the same and/or of different loci may independently contribute to the codification of a specific enzyme protein. In this case if the properties of the gene products differ in some respect, the outcome is enzyme polymorphism. Different forms of the same enzyme system are termed *allozymes* (or alloenzymes) when they are coded by allelic genes [1] and *isozymes* (or isoenzymes) when they are coded by non-allelic genes [2].

2. POTENTIALITIES FOR BASIC WORK AND FOR APPLICATIONS

Studies on enzyme polymorphisms have revealed many biological variabilities, undetectable by direct observational methods. They have also provided new dependable tools for research in various fields of biology.

Different enzyme forms generally can be recognized in both homozygous and heterozygous conditions, so that they are very suitable as markers in the analyses of genotypes [3] as well as in the study of developmental differentiation [4]. Some enzyme variants may cause physiological troubles, which could possibly be exploited for applied purposes. The so-called 'null mutants' deserve special attention. These can be detected when an expected enzyme activity is missing. The 'null mutants' may cause severe physiological deficiencies [5].

3. SENSITIVITY AND FEASIBILITY OF ELECTROPHORETIC METHODS

In work on electrophoretic enzyme polymorphisms two techniques are currently used: zone electrophoresis and isoelectrofocusing. Both these techniques enable enzyme molecules to be classified according to their electrophoretic mobility. The subsequent use of specific hystochemical stains reveals the reaction site of each molecular form as a stained band. The relative positions of the bands are proportional to the relative mobility of the enzyme molecules, and the staining intensities are related to enzyme activity levels. The measurement of activity levels requires the additional use of densitometric methods. Zone electrophoresis is technically easier; isoelectrofocusing has a much higher resolving power [6].

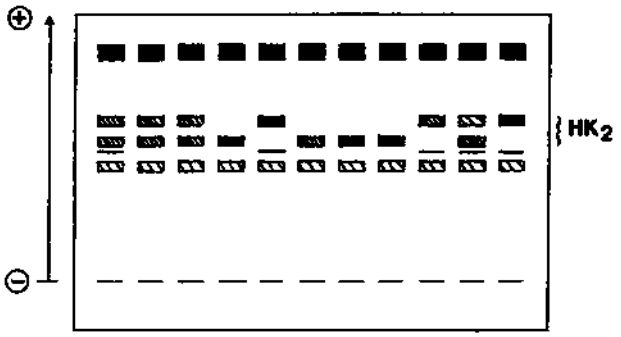

FIG.1. Zymograms of the HK system: HK_2 has two electrophoretic mobility phenotypes, both recognizable in the heterozygous phenotype.

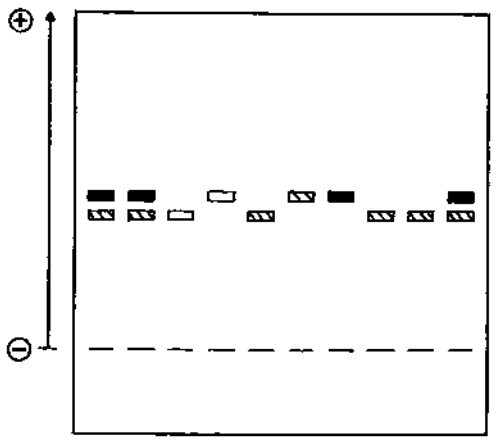

FIG.2. Zymograms of the MPI system: The band staining intensities are indicative of individual enzyme activity levels.

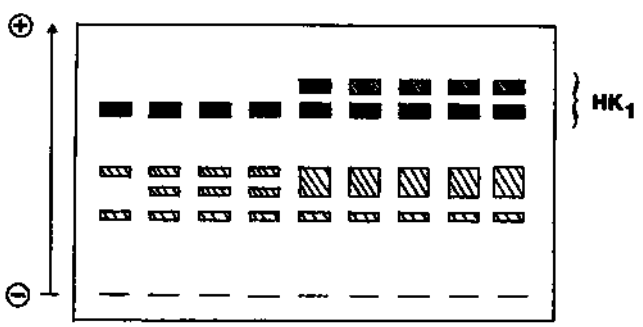

FIG.3. Zymograms of the HK system: The samples on the right show degradation of the HK_1 pattern due to 'storage' before electrophoretic separation.

4. PROBLEMS INVESTIGATED AND ENZYME SYSTEMS STUDIED

The number of forms which a single enzyme may present has been found generally high for enzymes having low substrate specificity (e.g. esterases, phosphatases); it has been found limited to a single or very few forms for those enzymes which require a specific substrate [7, 8]. Enzymes of glucose metabolisms belong to the second group.

In our studies on the medfly we have used zone electrophoresis. Attention has been focused on the enzymes that control glucose degradation and its various pathways. Such functions are of basic importance in energetic metabolism [9] and some variants of low or null activity could possibly be detected as potential candidates for use in genetic manipulations. This is not merely a theoretical assumption; for instance, drosophilas lacking α-GPDH (α-glycerol-3-phosphate dehydrogenase), an enzyme of the flight muscles, cannot fly [10]. We have screened eight medfly strains for 12 enzyme systems; some of these systems are represented by two or more isozymes, so the different enzyme functions so far examined amount to 17 (see Appendix). In all, this screening programme has covered 136 combinations, among which 34 have shown evidence of enzyme polymorphisms; these were due to electrophoretic mobility in 19 cases, to enzyme activity in five, and to both mobility and activity in 10. The results are summarized in Table I, and Figs 1–3 provide examples of variability for phenotypic enzyme properties.

The different forms of a given enzyme may show tissue and/or developmental stage specificity. As an example, the hexokinase system (HK) has four different functions: HK_1 is typical of thoracic muscles (and so of the imago); this function has given a single instance of polymorphism, with very low relative frequency of one variant. HK_2 is typical of the gut; it is polymorphic in all strains considered. HK_3 is typical of the ovary; it has always given a monomorphic picture. HK_4, found in the adult haemolymph and fat body, has a very limited level of variability. Tissue localization is shown in Fig.4, where zymograms obtained from single thoraxes or abdomens alternate.

The relations between tissue localization, function and degree of polymorphism observed in the HK system seem to parallel similar relations in other systems.

Mannose phosphate isomerase (MPI), which is described as a gut enzyme, is highly polymorphic in seven out of eight medfly strains (Table II), whereas α-GPDH, typical of the flight muscle, has provided only two instances of polymorphism, with low relative frequencies of one variant. Adenylate kinase (AK), which controls energy availability through the ATP ⇌ ADP equilibrium, has two isozymes, of different activity levels; the most active, and possibly physiologically most important [9], is monomorphic in all strains, whereas the one with lower activity has been found polymorphic in three out of eight strains (Fig.5).

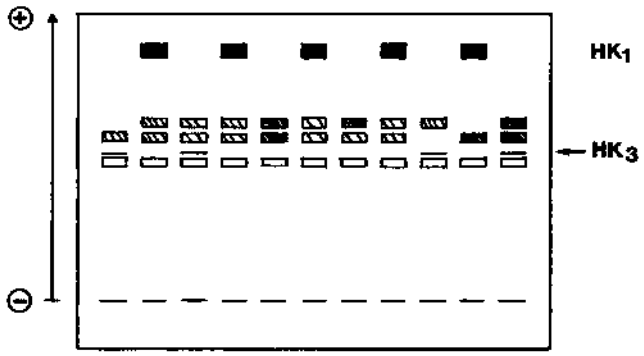

FIG.4. Zymograms of the HK system of individual abdomens and thoraxes alternate from the left: In all thorax preparations the HK_1 and HK_3 isozymes are respectively present and missing; HK_3 is also missing in the male abdomens' preparations.

TABLE II. THE GUT ENZYMES HK_2 AND MPI HAVE SHOWN POLYMORPHISM IN ALL BUT ONE EXAMINED STRAIN

The two HK_2 allozymes are regularly distributed, whereas the MPI-AA variant is generally missing

Enzymatic functions	HK_2			MPI		
Electrophoretic phenotypes	AA	AB	BB	AA	AB	BB
Italy	26	18	4	–	2	40
J.R.C. Ispra	40	69	34	5	26	4
Sardegna	14	31	12	–	46	34
Costa Rica	18	52	24	–	3	8
dark pupae	6	22	8	–	4	20
white pupae	16	28	5	–	–	88
orange eye	7	17	10	–	12	22
apricot eye	36	10	–	–	23	31

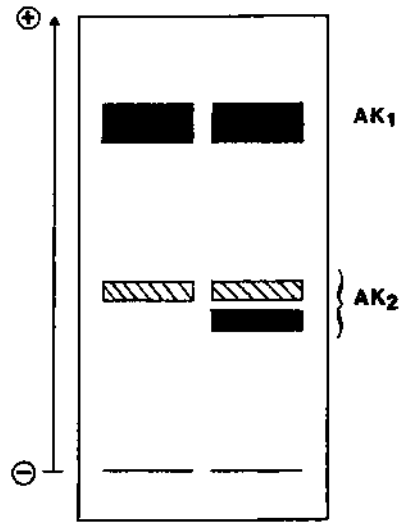

FIG.5. Zymograms of the AK system: AK_1 is monomorphic and produces a large intensely stained band; AK_2 is polymorphic and produces much thinner bands.

TABLE III. IN FAIRLY LARGE SAMPLES OF SOME STRAINS, ONE ALLOZYME HAS BEEN FOUND ONLY IN THE HETEROZYGOUS CONDITION

The sex distribution of the PGM_1-AA and PGM-AB phenotypes differ significantly from randomness

Enzyme function	Strain	Electrophoretic phenotypes		
		AA	AB	BB
AK_2	apricot eye	9	24	–
HK_2	apricot eye	36	10	–
MPI	Sardegna	–	46	34
MPI	orange eye	–	12	22
MPI	apricot eye	–	23	31
PGM_1	Sardegna	18.4 ♀ ♂ ($\chi^2 > 15$)	3.16 ♀ ♂	–

TABLE IV. GENE-ENZYME SYSTEMS IDENTIFIED IN *Ceratitis capitata* Wied.

Enzyme	Enzymatic function	Enzymatic structure	Genetic symbol	Mobility alleles	Additional phenotypic properties[a]
Adenylate kinase	AK_1[b]	–	–	–	–
	AK_2	Monomeric	Ak_2	2	
Hexokinase	HK_1	Monomeric	Hk_1	2	s
	HK_2	Monomeric	Hk_2	2	a
	HK_3[b]	–	–	–	–
	HK_4	Monomeric	Hk_4	2	
Glucose 6-phosphate dehydrogenase	G6PD	Dimeric (?)	*Zw*	2	
6-Phosphogluconate dehydrogenase	6PGD	Dimeric	*Pgd*	2	
Phosphoglucomutase	PGM_1	Monomeric	Pgm_1	2	a
α-Glycerophosphate dehydrogenase	α-GPDH	Dimeric	*Gpdh*	2	
Mannosephosphate isomerase	MPI	Monomeric	*Mpi*	2	a
Xanthine dehydrogenase	XDH	Dimeric	*Xdh*	2	

[a] a = activity; s = stability.

[b] Monomorphic function.

All strains have shown at least one polymorphic enzyme system, with conspicuous differences between strains. The lowest variability has been found in the strains J.R.C. Ispra, dark pupae, white pupae; in these three strains enzyme variability is limited to the gut enzymes. The greatest variability amounts to nine polymorphic enzyme functions which have been observed on samples of the second laboratory generation of the Sardegna strain.

The relative frequencies of the various enzyme forms generally do not differ between sexes. The only exception in our records comes from a single observation on function PGM_1 in the Sardegna strain, based on 41 flies, and was associated with absence of one homozygous class of flies. Also in other instances one of the two variants has never been found in the homozygous condition (Table III). Variants with similar relative frequencies and regular distribution among all expected phentotypes have been observed for the HK_2 function.

An overall picture of the main results so far obtained is given in Table IV.

By proper statistical treatment, the data already available should provide clues for the detection of genetic linkage between some of the variants so far studied.

The next step in our research programme will be a search for linked loci, as a preliminary to the study of recombination and of sex determination in the medfly.

APPENDIX

List of strains

Italy; J.R.C. Ispra; Sardegna; Costa Rica; dark pupae; white pupae; orange eye; apricot eye.

List of enzyme functions

AK_1 AK_2 (adenylate kinase); HK_1 HK_2 HK_3 HK_4 (hexokinase); G6PD (glucose 6-phosphate dehydrogenase); 6PGD (6-phosphogluconate dehydrogenase); PGM_1 PGM_2 (phosphoglucomutase); α-GPDH (α-glycerol-3-phosphate dehydrogenase); MPI (mannosephosphate isomerase); XDH (xantine dehydrogenase); PGI (phosphoglucose isomerase); FH (fumarase); HAD (β-hydroxyacid dehydrogenase); GOX (glucose oxidase).

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A PLAN TO CONSTRUCT THE NEW MASS PRODUCTION FACILITY FOR THE MELON FLY, *Dacus cucurbitae* Coquillett, IN OKINAWA, JAPAN

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Abstract

A PLAN TO CONSTRUCT THE NEW MASS PRODUCTION FACILITY FOR THE MELON FLY, *Dacus cucurbitae* Coquillett, IN OKINAWA, JAPAN.

In 1980, the Japanese Ministry of Agriculture, Forestry and Fisheries, and the Prefectural Government of Okinawa, began a large-scale project aimed to eradicate by means of the sterile insect technique (SIT) the melon fly from the whole of the Okinawa Prefecture. For this project, a new mass production facility producing 100 million pupae of the melon fly weekly is being constructed. The facility is a three-storeyed building with a total floor area of 4265.6 m^2. The facility is divided into two parts, open and closed areas, to prevent the escape of the reared fertile melon flies from the facility. Most of the mass production rooms are in the closed area. Adults are reared in two adult rearing rooms and the average number of eggs collected from these rooms is 156 million weekly. Eggs are seeded on larval media every Tuesday, Wednesday, Thursday and Friday in each room and these four series of rearings finish on the eighth day after seeding. Mature larvae are placed in a plastic tray with sawdust for pupation. Pupae are sifted on the fifth day after pupation to prevent the droopy-wing syndrome. The automatic conveyor systems are adopted for larval rearing, pupation and sifting. There are four pupal store rooms, the room temperature of which can be controlled in the range $15 \pm 1^{\circ}$ to $27 \pm 1^{\circ}$C. The speed of pupal development from different batches can be adjusted by the room temperature. By this adjustment, pupae of a constant amount can be irradiated and released every day. Quality control tests are regularly conducted in the quality control rooms in the second storey.

INTRODUCTION

The Okinawa Prefecture, Japan, consists of the Okinawa Islands (143 298 ha), the Miyako Islands (22 696 ha) and the Yaeyama Islands (58 442 ha). The melon fly, *Dacus cucurbitae*, is distributed throughout Okinawa prefecture with the exception of Kume Island (6247 ha) where the fly was eradicated in 1977 by means of the sterile insect technique (SIT) [1].

In 1980, the Japanese Ministry of Agriculture, Forestry and Fisheries and the Prefectural Government of Okinawa began a large-scale project aimed at

TABLE I. YEARLY SCHEDULE OF THE PROJECT TO ERADICATE THE MELON FLY IN OKINAWA PREFECTURE, JAPAN

Year	Item
1980	Construction of the building for mass production (total floor capacity 100 million pupae/week)
1981–82	Construction of the equipment for producing 30 million pupae/week
1983	Construction of the irradiation facility (for sterilizing 100 million pupae/week)
1984–86	Release of sterile flies and eradication in Miyako Islands (22 696 ha)
1986	Reinforcement of the equipment to produce 100 million pupae/week
1987–89	Release of sterile flies and eradication in Okinawa Islands (143 298 ha)
1990–91	Release of sterile flies and eradication in Yaeyama Islands (58 442 ha)

eradicating the melon fly from the whole of Okinawa prefecture with SIT. In the course of this project, a new mass production facility producing 100 million melon fly pupae weekly is being constructed. The plan to construct this facility is described here.

YEARLY SCHEDULE TO CONSTRUCT THE MASS PRODUCTION FACILITY

Table I shows a yearly schedule of the project. According to this schedule, construction of the mass production facility producing 30 million pupae weekly will be finished in 1982, and later it will be developed so as to produce 100 million pupae weekly in 1986. The facility in its final mode will be described.

OUTLINE OF THE MASS PRODUCTION FACILITY

The facility is a three-storeyed building with a total floor area of 4265.6 m^2. A floor plan of the facility is shown in Fig. 1. The first storey consists of office, larval rearing and machinery sections. Pupal rearing and quality control sections are located in the second storey. In the third storey, the adult rearing and the new strain colonization sections are placed. In the planning of the facility,

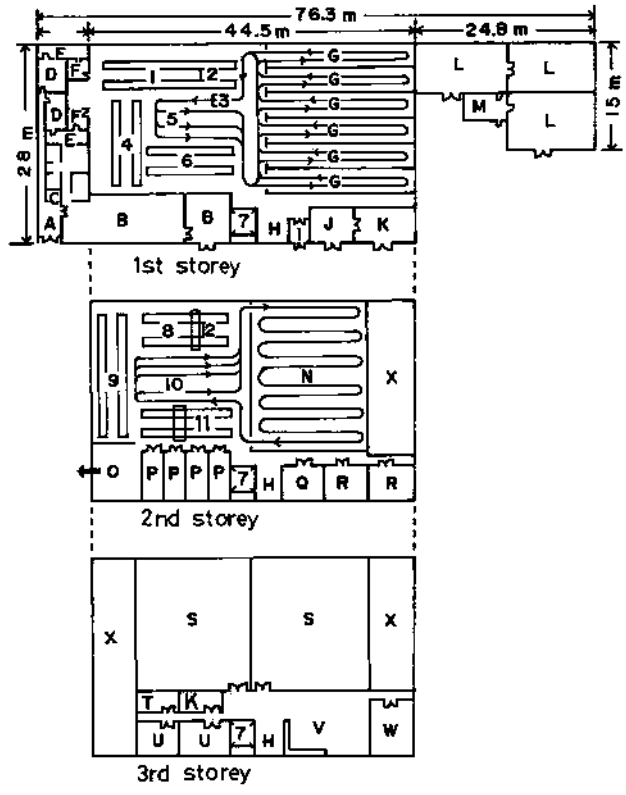

FIG.1. Plan of the mass production facility. A: Entrance; B: Office and council room; C: Guard room; D: Locker room; E: Shower stall; F: Dressing room; G: Larval-rearing room; H: Staircase; I: Hot room; J: Larval diet mixing room; K: Store room; L.: Machinery room; M: Operation room; N: Pupation room; O: Pupae-loading room; P: Pupae-storing room; Q: Maintenance room; R: Quality-control room; S: Adult-rearing room; T: Preparation room; U: New strain colonization room; V: Egg collection and adult cage washing room; W: Dining and rest room; X: Air-conditioner.- 1: Tray unloader and washing device; 2: Larvae-collection device; 3: Frame washing; 4: Tray stock; 5: Frame stock; 6: Diet, egg supply and tray loading device; 7: Lift; 8: Sawdust and larvae supply device; 9: Tray stock; 10: Frame stock; 11: Sifting device.

quality control, prevention of escape of the fertile fly, automation of rearing procedure, saving water by a recycling system and purification of air polluted by odour from rearing media, were given particular attention.

System to prevent the escape of the flies reared

The facility is divided into two parts, open and closed areas, to prevent the escape of the reared fertile melon fly from the facility. The open area consists

TABLE II. MASS REARING SCHEDULE OF THE MELON FLY IN THE FACILITY PRODUCING 100 MILLION PUPAE WEEKLY[a]

Larval room No.	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.	Sun.	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.	Sun.
	A-1	EC								A-1	EC			
I.		ES	L	L	L	L	LC	LC	LC	W	ES	L	L	L
		A-2	EC								A-2	EC		
II.			ES	L	L	L	L	LC	LC	LC	W	ES	L	L
			A-1	EC										
III.				ES	L	L	L	L	LC	LC	LC	W		
				A-2	EC									
IV.					ES	L	L	L	L	LC	LC	LC	W	
								A-1	EC					
V.									ES	L	L	L	L	LC
									A-2	EC				
VI.										ES	L	L	L	L

[a] A-1: Insertion of egging device in adult room 1.
A-2: Insertion of egging device in adult room 2.
EC: Egg collection.
ES: Egg seeding on the larval media.
L: Larval rearing.
LC: Larval collection.
W: Washing of larval rearing room.

of office, machinery rooms, the larval diet mixing room, etc. Most of the mass production rooms and laboratories for quality control are in the closed area. Workers are obliged to change clothes when entering and leaving the closed area. The larval media are mixed in the larval diet mixing room in the open area and are pumped into the closed area. The remnant of the culture media is pumped out after treating with boiling water. Machines for mass rearing are maintained inside the closed area. When it is necessary to remove anything from the closed area, it must pass through the hot room where any flies adhering to it are killed by high temperature. All pupae produced are brought out through the irradiation facility.

Adult rearing

Adults are reared in two adult rearing rooms in which the air temperature is 26 ± 2°C. An adult room accommodates 240 adult cages, in each of which 50 000 adults are reared [2]. Eggs are collected twice a week in each room with an egging device. The average number of eggs collected from two adult rooms is 156 million weekly. Both a fluorescent lamp and natural light from windows on the ceiling light the adult rooms at an intensity of about 1000 lux during the day. At dawn or dusk the number of fluorescent lamps in use is increased or decreased automatically. This light-controlling procedure aims to maintain the diurnal behavioural rhythms of the reared flies as near as possible to those of wild ones. However, flies are lighted 24 hours on the day of egg collection to stimulate egg laying.

Larval rearing

The mass rearing schedule of the melon fly in the facility is shown in Table II. For this schedule it needs six larval rearing rooms, each having a capacity to produce 31.2 million mature larvae. Eggs are seeded on larval media every Tuesday, Wednesday, Thursday and Friday in each room and these four series of rearings finish on the eighth day after seeding. The temperature of the larval rearing room can be controlled over a range from 20 ± 1° to 27 ± 1°C, depending on larval maturation [2]. Mature larvae of the melon fly pop out of the media from the fifth to the seventh day after seeding. This larvae are gathered with water and conveyed to the second storey. The following two automatic conveyor systems are adopted for larval rearing. (1) Supply of larval medium on a tray, eggs seeding on the medium, tray loading on a frame and distribution of the frame to each larval room. (2) Frame taken from the larval room, unloading the tray from the frame, washing and storing the tray.

Pupal rearing

Pupal rearing is conducted in the second storey. Mature larvae are separated from the water and placed on a plastic tray with sawdust. The sawdust is stored in the closed hopper located outside the facility and is brought into the facility by a conveyor. There is a pupation room having a rearing capacity of 100.3 million pupae, the temperature of this room being maintained at 25 ± 1°C. Under these conditions, pupae are sifted at on the fifth day after pupation to prevent the droopy-wing syndrome [3]. Automatic conveyor systems are also adopted for pupation and sifting. There are four pupae storing rooms, the room temperature of which can be controlled over a range from 15 ± 1° to 27 ±1°C. Various pupal developmental stages in different cultural series can be adjusted by the temperature

of the pupae storing rooms. By this adjustment, pupae of a constant amount can be irradiated and released every day.

Quality control

Quality control tests are conducted regularly in the quality control rooms in the second storey. In these tests many of the insects produced are sampled and the number of eggs per adult cage, the percentage of egg hatch, the number of mature larvae that pupated successfully, pupal size, adult emergence rate, etc., are determined. Flight-mill and actograph tests are also conducted in these rooms.

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The following conversion table is provided for the convenience of readers

FACTORS FOR CONVERTING SOME OF THE MORE COMMON UNITS TO INTERNATIONAL SYSTEM OF UNITS (SI) EQUIVALENTS

NOTES:

(1) SI base units are the metre (m), kilogram (kg), second (s), ampere (A), kelvin (K), candela (cd) and mole (mol).

(2) ▶ indicates SI derived units and those accepted for use with SI;
▷ indicates additional units accepted for use with SI for a limited time.
[*For further information see the current edition of The International System of Units (SI), published in English by HMSO, London, and National Bureau of Standards, Washington, DC, and International Standards ISO-1000 and the several parts of ISO-31, published by ISO, Geneva.*]

(3) The correct symbol for the unit in column 1 is given in column 2.

(4) * indicates conversion factors given exactly; other factors are given rounded, mostly to 4 significant figures:
≡ indicates a definition of an SI derived unit; [ ] in columns 3+4 enclose factors given for the sake of completeness.

Column 1 *Multiply data given in:*	Column 2	Column 3 *by:*	Column 4 *to obtain data in:*	
Radiation units				
▶ becquerel	1 Bq	(has dimensions of s^{-1})		
disintegrations per second (= dis/s)	1 s^{-1}	≡ 1.00×10^{0}	Bq	*
▷ curie	1 Ci	= 3.70×10^{10}	Bq	*
▷ roentgen	1 R	[= 2.58×10^{-4}	C/kg]	*
▶ gray	1 Gy	[≡ 1.00×10^{0}	J/kg]	*
▷ rad	1 rad	= 1.00×10^{-2}	Gy	*
▶ sievert *(radiation protection only)*	1 Sv	[= 1.00×10^{0}	J/kg]	*
rem *(radiation protection only)*	1 rem	[= 1.00×10^{-2}	J/kg]	*
Mass				
▶ unified atomic mass unit ($\frac{1}{12}$ of the mass of ^{12}C)	1 u	[= $1.660\,57 \times 10^{-27}$	kg, approx.]	
▶ tonne (= metric ton)	1 t	[= 1.00×10^{3}	kg]	*
pound mass (avoirdupois)	1 lbm	= 4.536×10^{-1}	kg	
ounce mass (avoirdupois)	1 ozm	= 2.835×10^{1}	g	
ton (long) (= 2240 lbm)	1 ton	= 1.016×10^{3}	kg	
ton (short) (= 2000 lbm)	1 short ton	= 9.072×10^{2}	kg	
Length				
statute mile	1 mile	= 1.609×10^{0}	km	
nautical mile (international)	1 n mile	= 1.852×10^{0}	km	*
yard	1 yd	= 9.144×10^{-1}	m	*
foot	1 ft	= 3.048×10^{-1}	m	*
inch	1 in	= 2.54×10^{1}	mm	*
mil (= 10^{-3} in)	1 mil	= 2.54×10^{-2}	mm	*
Area				
▷ hectare	1 ha	[= 1.00×10^{4}	m^2]	*
▷ barn *(effective cross-section, nuclear physics)*	1 b	[= 1.00×10^{-28}	m^2]	*
square mile, (statute mile)^2	1 $mile^2$	= 2.590×10^{0}	km^2	
acre	1 acre	= 4.047×10^{3}	m^2	
square yard	1 yd^2	= 8.361×10^{-1}	m^2	
square foot	1 ft^2	= 9.290×10^{-2}	m^2	
square inch	1 in^2	= 6.452×10^{2}	mm^2	
Volume				
▶ litre	1 l *or* 1 ltr	[= 1.00×10^{-3}	m^3]	*
cubic yard	1 yd^3	= 7.646×10^{-1}	m^3	
cubic foot	1 ft^3	= 2.832×10^{-2}	m^3	
cubic inch	1 in^3	= 1.639×10^{4}	mm^3	
gallon (imperial)	1 gal (UK)	= 4.546×10^{-3}	m^3	
gallon (US liquid)	1 gal (US)	= 3.785×10^{-3}	m^3	

Column 1 *Multiply data given in:*	Column 2	Column 3 *by:*	Column 4 *to obtain data in:*	
Velocity, acceleration				
foot per second (= fps)	1 ft/s	= 3.048×10^{-1}	m/s	*
foot per minute	1 ft/min	= 5.08×10^{-3}	m/s	*
mile per hour (= mph)	1 mile/h	= 4.470×10^{-1} = 1.609×10^{0}	m/s km/h	
▷ knot (international)	1 knot	= 1.852×10^{0}	km/h	*
free fall, standard, g		= 9.807×10^{0}	m/s^2	
foot per second squared	1 ft/s^2	= 3.048×10^{-1}	m/s^2	*
Density, volumetric rate				
pound mass per cubic inch	1 lbm/in^3	= 2.768×10^{4}	kg/m^3	
pound mass per cubic foot	1 lbm/ft^3	= 1.602×10^{1}	kg/m^3	
cubic feet per second	1 ft^3/s	= 2.832×10^{-2}	m^3/s	
cubic feet per minute	1 ft^3/min	= 4.719×10^{-4}	m^3/s	
Force				
▶ newton	1 N	[≡ 1.00×10^{0}	$m \cdot kg \cdot s^{-2}$]	*
dyne	1 dyn	= 1.00×10^{-5}	N	*
kilogram force (= kilopond (kp))	1 kgf	= 9.807×10^{0}	N	
poundal	1 pdl	= 1.383×10^{-1}	N	
pound force (avoirdupois)	1 lbf	= 4.448×10^{0}	N	
ounce force (avoirdupois)	1 ozf	= 2.780×10^{-1}	N	
Pressure, stress				
▶ pascal	1 Pa	[≡ 1.00×10^{0}	N/m^2]	*
▷ atmosphere[a], standard	1 atm	= $1.013\,25 \times 10^{5}$	Pa	*
▷ bar	1 bar	= 1.00×10^{5}	Pa	*
centimetres of mercury (0°C)	1 cmHg	= 1.333×10^{3}	Pa	
dyne per square centimetre	1 dyn/cm^2	= 1.00×10^{-1}	Pa	*
feet of water (4°C)	1 ftH_2O	= 2.989×10^{3}	Pa	
inches of mercury (0°C)	1 inHg	= 3.386×10^{3}	Pa	
inches of water (4°C)	1 inH_2O	= 2.491×10^{2}	Pa	
kilogram force per square centimetre	1 kgf/cm^2	= 9.807×10^{4}	Pa	
pound force per square foot	1 lbf/ft^2	= 4.788×10^{1}	Pa	
pound force per square inch (= psi)[b]	1 lbf/in^2	= 6.895×10^{3}	Pa	
torr (0°C) (= mmHg)	1 torr	= 1.333×10^{2}	Pa	
Energy, work, quantity of heat				
▶ joule (≡ W·s)	1 J	[≡ 1.00×10^{0}	N·m]	*
▶ electronvolt	1 eV	[= $1.602\,19 \times 10^{-19}$	J, approx.]	
British thermal unit (International Table)	1 Btu	= 1.055×10^{3}	J	
calorie (thermochemical)	1 cal	= 4.184×10^{0}	J	*
calorie (International Table)	1 cal_{IT}	= 4.187×10^{0}	J	
erg	1 erg	= 1.00×10^{-7}	J	*
foot-pound force	1 ft·lbf	= 1.356×10^{0}	J	
kilowatt-hour	1 kW·h	= 3.60×10^{6}	J	*
kiloton explosive yield (PNE) (≡ 10^{12} g-cal)	1 kt yield	≃ 4.2×10^{12}	J	

[a] atm (g) (= atü): atmospheres gauge
atm abs (= ata): atmospheres absolute

[b] lbf/in^2 (g) (= psig): gauge pressure;
lbf/in^2 abs (= psia): absolute pressure.

Column 1 *Multiply data given in:*	Column 2	Column 3 *by:*	Column 4 *to obtain data in:*	
Power, radiant flux				
▶ watt	1 W	[≡ 1.00×10^{0}	J/s]	*
British thermal unit (International Table) per second	1 Btu/s	= 1.055×10^{3}	W	
calorie (International Table) per second	1 cal_{IT}/s	= 4.187×10^{0}	W	
foot-pound force/second	1 ft·lbf/s	= 1.356×10^{0}	W	
horsepower (electric)	1 hp	= 7.46×10^{2}	W	*
horsepower (metric) (= ps)	1 ps	= 7.355×10^{2}	W	
horsepower (550 ft·lbf/s)	1 hp	= 7.457×10^{2}	W	
Temperature				
▶ kelvin	K			
▶ degrees Celsius, t where T is the thermodynamic temperature in kelvin and T_0 is defined as 273.15 K	$t = T - T_0$			*
degree Fahrenheit	$t_{°F} - 32$	$\times \left(\frac{5}{9}\right)$ gives	t *(in degrees Celsius)*	*
degree Rankine	$T_{°R}$		T *(in kelvin)*	*
temperature difference[c]	$\Delta T_{°R}$ (= $\Delta t_{°F}$)		ΔT (= Δt)	*
Thermal conductivity [c]				
1 Btu·in/(ft²·s·°F) *(International Table Btu)*		= 5.192×10^{2}	$W \cdot m^{-1} \cdot K^{-1}$	
1 Btu/(ft·s·°F) *(International Table Btu)*		= 6.231×10^{3}	$W \cdot m^{-1} \cdot K^{-1}$	
1 cal_{IT}/(cm·s·°C)		= 4.187×10^{2}	$W \cdot m^{-1} \cdot K^{-1}$	
Miscellaneous quantities				
litre per mole per centimetre (1M/cm =) *(molar extinction coefficient or molar absorption coefficient)*	1 $ltr \cdot mol^{-1} \cdot cm^{-1}$	= 1.00×10^{-1}	m^2/mol	*
G-value, traditionally quoted per 100 eV of energy absorbed *(radiation yield of a chemical substance)*	1×10^{-2} eV^{-1}	= 6.24×10^{16}	J^{-1}	
mass per unit area *(absorber thickness and mean mass range)*	1 g/cm²	[= 1.00×10^{1}	kg/m^2]	*

[c] A temperature interval or a Celsius temperature difference can be expressed in degrees Celsius as well as in kelvins.

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