Sterile-Male Technique for Control of Fruit Flies

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1970

STERILE-MALE TECHNIQUE
FOR CONTROL OF FRUIT FLIES

.

.

STERILE-MALE TECHNIQUE FOR CONTROL OF FRUIT FLIES

PROCEEDINGS OF A PANEL
ON THE APPLICATION
OF THE STERILE-MALE TECHNIQUE
FOR CONTROL OF INSECTS
WITH SPECIAL REFERENCE TO FRUIT FLIES

ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE
AND HELD IN VIENNA, 1 – 5 SEPTEMBER 1969

STERILE-MALE TECHNIQUE FOR CONTROL OF FRUIT FLIES
IAEA, VIENNA, 1970
STI/PUB/276

FOREWORD

Previous panels on the application of the sterile-male technique to insects have resulted in the following Agency publications in the Technical Reports Series (TRS) and the Panel Proceedings Series (PPS):

TRS 21: Insect population control by the sterile-male technique (1963)
TRS 44: Advances in insect population control by the sterile-male technique (1965)
PPS: Radiation, radioisotopes and rearing methods in the control of insect pests (1968)
PPS: Control of livestock insect pests by the sterile-male technique (1968)
PPS: Insect ecology and the sterile-male technique (1969)
PPS: Sterile-male technique for eradication or control of harmful insects (1969).

The present panel, on the Application of the Sterile-Male Technique for Control of Insects, with Special Reference to Fruit Flies, was held by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture in Vienna on 1 - 5 September 1969.

Among the many noxious pest species, fruit flies of the family Tephritidae are specially suitable for control or eradication by means of the sterile-male technique since they are rather easily reared in great numbers at a relatively low cost. This explains why more projects have been set up to prove the feasibility of the technique applied to fruit flies than for any other group of insect pests, and many developing countries are planning to examine the practical application of the technique for some of their fruit-fly species.

Although a few years ago it was thought that the sterile-male technique as applied to fruit flies was simple, the Agency's cooperative research projects in Central America, Italy and Spain have shown that a vast amount of data on basic biology and field ecology of sterile and wild fruit flies is needed before this method of control or eradication can be applied on a large scale. Some species, such as the Mediterranean fruit fly, the Mexican fruit fly and others belonging to the genus Anastrepha, and the melon fly, can be mass-reared at a relatively low cost, but complete mechanization has to be achieved before large-scale eradication campaigns can be started. For some species, such as the olive fruit fly, mass rearing remains a problem, and for several other species many of the basic biological and ecological data, as well as sterilizing dosages, mass-rearing techniques, etc., are still missing.

The present publication contains the formal papers presented at the panel meeting together with some short contributions summarizing and surveying recent work at various centres. Because many of the papers deal with the Mediterranean fruit fly, these have been put together in a separate section. A section setting out a number of general and specific

recommendations is also included. For those working in close touch on this subject with the Joint FAO/IAEA Division and for scientists in many countries engaged in fruit-fly eradication or control programs using the sterile-male technique these recommendations form a very important part of the book. They are intended to be a guide to the Joint Division in planning the future programs of the Division's Insect Eradication and Pest Control Section.

CONTENTS

FRUIT FLIES OTHER THAN MEDITERRANEAN FRUIT FLY:
  SHORT CONTRIBUTIONS

RECOMMENDATIONS

MEDITERRANEAN FRUIT FLY:
REPORTS

.

STERILIZATION OF
THE MEDITERRANEAN FRUIT FLY

A review of laboratory data

G.H.S. HOOPER
International Atomic Energy Agency,
Vienna

Abstract

STERILIZATION OF THE MEDITERRANEAN FRUIT FLY: A REVIEW OF LABORATORY DATA.
The current status of research on the sterilization of Ceratitis capitata (Wiedemann) is reviewed.
Most of the paper is devoted to gamma radiation induced sterility, but the available information on the
use of neutrons and chemicals to induce sterility is also included.

1. INTRODUCTION

In the thirty odd years which have passed since Knipling conceived
the idea of controlling injurious insect species by means of reproductive
sterility, there has been considerable interest in, and research on,
this technique. As work progressed it became very evident that while
the sterile-male technique is attractively simple in theory, it is in fact
very sophisticated, requiring basic data on the biology of the insect
being investigated. The agents used to produce sexual sterility, be
they radiations or chemosterilants, are not specific for the reproductive
system. Their effects on other relevant biological characteristics must
be evaluated in order to produce not only a sterile male, but a healthy,
sexually aggressive male.

In this paper an attempt is made to review the current status of
research on the sterilization of the Mediterranean fruit fly, Ceratitis
capitata (Wiedemann), under various headings. Most of the paper deals
with sterilization by gamma irradiation, but the available information
on neutron irradiation and chemosterilization is also included.

2. ADULT EMERGENCE AND SURVIVAL

While no emergence occurred when 5 - 10 krad were given to pupae
5 days before emergence, better than 50% emergence was obtained with
the same dosages given one day later. Once pupae were within 3 days
of emergence, dosages up to 20 krad had no adverse effect (Katiyar (1962),
Katiyar and Valerio (1964a), Feron (1966), Shoukry (1968)). Beyond
20 krad emergence was progressively decreased and ceased with
dosages greater than 80 krad (Fig.1).

The survival of flies irradiated at -2 days (i.e. pupae irradiated
2 days before adult emergence) was not reduced by dosages up to
13 krad over a period of 6 - 7 weeks (Katiyar (1962)). Later work from
his laboratory showed that the longevity of neither sex was adversely
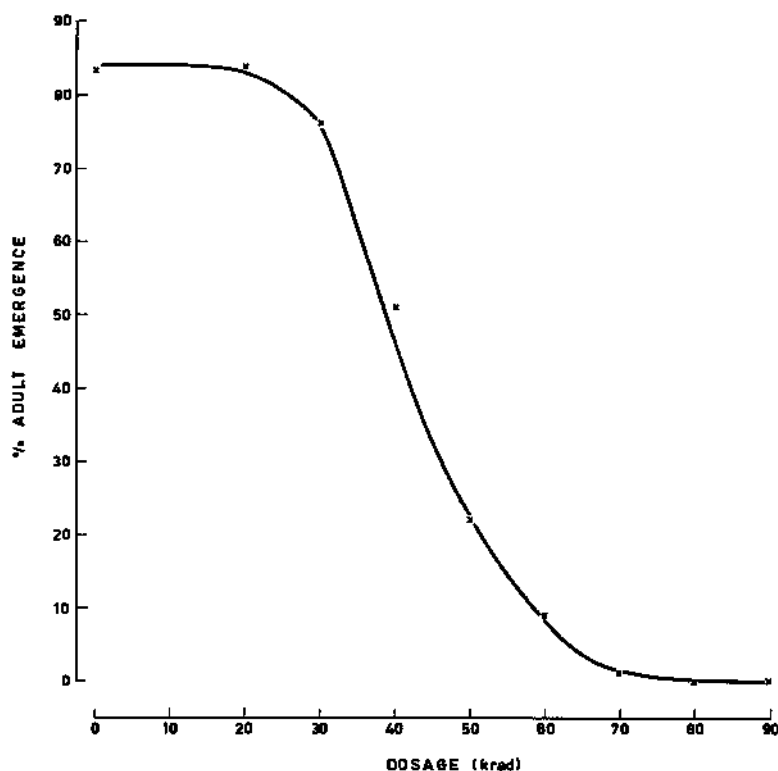
FIG.1. Percent adult emergence from pupae treated 3 days before emergence with gamma radiation. (Data from Katiyar and Valerio (1964a)).

affected by dosages up to 20 krad (at -3 days). Beyond 20 krad (Fig.2), mortality progressively increased (Katiyar and Valerio (1964a)). However, with dosages up to 20 krad applied at -2 days, Keiser and Schneider (1969b) found that lower dosages (2.5 - 10 krad) increased female longevity while dosages of 15 and 20 krad decreased male longevity. On the other hand, Feron (1966) found that a dosage of 15 krad (at -3 to -2 days) caused rapid mortality, particularly of males which survived only a few days. Feron also found that while 5 krad had no adverse effect, increasing the dosage to 10 krad progressively decreased longevity. Similarly, Steiner and Christenson (1956) reported that above 8.5 krad mortality of the emergent flies increased.

In the above experiments food and water were provided for the emerging flies. Recent studies in Hawaii showed that when deprived of food and water, newly emerged irradiated flies (10 krad at -2 days) survived as well as untreated flies. However, after about 50% of the population had died naturally, there were indications that irradiated flies withstood food and water deprivation better than did untreated flies (Keiser and Schneider (1969a, b)).
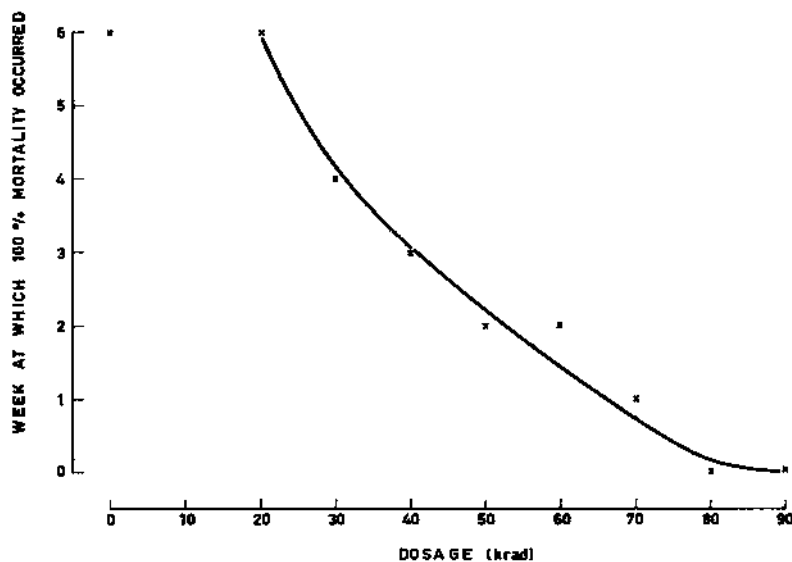
FIG.2. Survival of adults which emerged from pupae treated 8 days before emergence with gamma radiation. (Data from Katiyar and Valerio (1964a)).
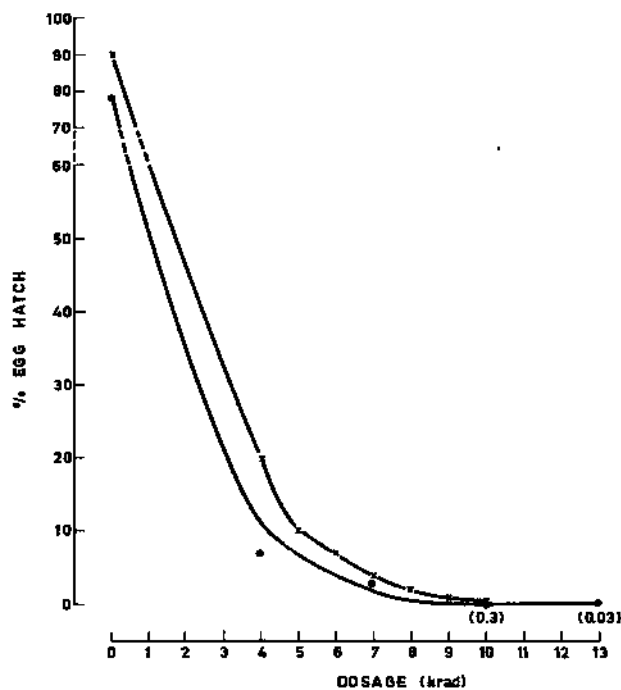
FIG.3. Relationship between hatch of eggs from untreated females mated with males treated with gamma radiation. (Data from Katiyar (1962) and Katiyar and Ramirez (1969)).

On the relationship between age of the pupae when irradiated and subsequent adult survival, there is only one piece of evidence. Arroyo et al. (1965a) found that, after a dosage of 8 krad, the longevity of adults was greater when they were irradiated at -3 days than at -4 or -5 days.

3. STERILITY IN MALES

In all the work reported, male sterility has been assessed by recording the hatch of eggs resulting from the mating of irradiated males with untreated females. In C. capitata this criterion of sterility is acceptable since Feron (1966) found that no mortality occurred after larval eclosion.

All workers have found that the degree of sterility produced in males is proportional, though not linearly, to dosage (e.g. Steiner and Christenson (1956), Katiyar and Ramirez (1969)). This situation is illustrated in Fig.3 with data from Katiyar (1962) and Katiyar and Ramirez (1969). Once sterility of approximately 95% is achieved, large increases in dosage are required to produce relatively small increases in sterility.

With respect to pupal age at irradiation, Katiyar and Valerio (1964a) found little difference in sterility between males irradiated at -1, -2 or -3 days. While this is certainly true for dosages of 7.5 and 10 krad, there is the suggestion in their data that at 5 krad the degree of sterility decreased as the time of irradiation approached the end of pupal life.

In Table I an attempt has been made to summarize dosage-sterility data in the literature. There is fairly good agreement that to ensure egg hatch of less than 0.5% a dosage of about 10 krad is required.

TABLE I. STERILITY OF MALE C. capitata INDUCED BY GAMMA RADIATION
Sterility was assessed by hatch of eggs resulting from the cross-irradiated male X untreated female

Age at which males irradiated (days)	Dosage (krad) required for sterility equal to, or better than:		Reference
	95%	99.5%	
-2	7	10	Katiyar (1962)
-1	5	10	Katiyar & Valerio (1964a)
-2	7.5	10	'' '' ''
-3	7.5	10	'' '' ''
-2	7	10	Feron (1966)
-2 to -3	4	6	Shoukry (1968)
-1	6	9	Schemey & Haisch (1968)
-2	7	11	Hooper (unpublished data)

It has been reported from Hawaii that dosages of 8.4 krad or lower permitted a substantial increase in fertility in males after 30 - 50 days (Steiner and Christenson (1956), Steiner et al. (1962)). However, these papers deal with two other Tephritid species as well as C. capitata and it is not clear whether the above conclusion is true of all three species.

Feron (1966) claimed an occasional recovery of fertility, based on egg hatch, in males after 20 - 25 days on the basis of results from a 64 : 1 ratio test involving males treated with 8 krad at -3 to -2 days. However, his data could equally well be explained by (a) higher mortality of treated than untreated males or (b) sperm depletion in the treated males. In both situations an increase in effective matings of females by untreated males would occur which would lead to increased egg hatch. Furthermore, recovery of fertility should not be assessed in a competition test.

The data of Arroyo et al. (1965a) are confusing. Whilst these authors state in their discussion that egg hatch increased after about 2 weeks, following treatment of males with 10 and 15 krad (at -4 days), in the appropriate tables presenting the results it is specifically stated that no egg hatch occurred during the experiment.

Katiyar's group has not reported any loss of sterility in males and none of our work suggests that recovery of fertility occurs (Hooper, unpublished data). Histological studies by Causse et al. (1968) argue against the recovery of fertility in irradiated males noted above. It was found that at 4 and 5 krad (given at -2 days) the whole of the apical region of the follicles was disorganized in individuals aged 4 days. One month after a dosage of 9 krad the testes were atrophied.

4. STERILITY IN FEMALES

Female C. capitata are more sensitive to gamma radiation than males [Feron and Serment (1963) stated the reverse but this was obviously an error as their own later work (Feron (1966)) showed]. The increased sensitivity of the female may be explained by the fact that the ovaries show little development in 8-day-old pupae whereas in males, spermatogenesis is well advanced and all developmental stages are present (Causse et al. (1968)).

Females irradiated at -3 to -1 days produced eggs after dosages of 1 and 2 krad. At 3 krad egg production was irregular and above 4 krad very few if any eggs were produced (Katiyar (1962), Feron (1966), Shoukry (1968)). However, Steiner et al. (1962) found that at a dosage of 8.5 krad females did produce some fertile eggs, and Katiyar and Valerio (1964a) reported that 7.5 krad (at -1 day) allowed some egg production. We have found that females irradiated with dosages as high as 13 krad (at -2 days) produced a few eggs (Hooper, unpublished data) but none hatched. Since it is hatched eggs which are of importance in a sterile-insect release experiment, this phenomenon is of little practical importance.

Feron (1966) found that the hatch of eggs produced by females given 1, 2 and 3 krad (at -3 to -2 days) was reduced; the corrected egg hatch was 65, 56 and 15% respectively. In an admittedly limited test we found no reduction in egg hatch at 1 and 2 krad (Hooper, unpublished data).

        As pupal development proceeds higher dosages are required to
produce infecundity. Thus Katiyar and Valerio (1964a) found that while
5 krad at -2 days produced infecundity, one day later 10 krad was
required.
        It is not unequivocally resolved whether the fecundity of an un-
treated female is lowered after mating with an irradiated male. Re-
ferring to the work of Steiner and Christenson (1956), Lindquist (1963)
stated that fecundity was decreased. Arroyo et al. (1965a, b) made
similar observations. On the other hand, the present author (Hooper,
unpublished data) found no differences in the mean female fecundity,
over a period of 11 days, following the mating of untreated females
with males treated with 7, 5, 9, 10 or 12 krad at -2 days.

5. EFFECT OF IRRADIATION ON MATING CHARACTERISTICS

(a) Male competitiveness

        Apart from the induction of an acceptable degree of sterility, it is
of the utmost importance that the irradiated male be as sexually com-
petitive, vis-à-vis the wild male, as possible. In the laboratory the
competitiveness of the treated male is compared with that of the un-
treated male. This is not the same thing, but is as much as is usually
possible.
        As a generalization it can be said that competitiveness of the male
is inversely proportional to dosage (Steiner and Christenson (1956))
and directly proportional to age of the pupa when irradiated
(Katiyar et al. (1966)).
        Competitiveness has been evaluated in three ways: (i) by comparing
the number of observed matings, (ii) by comparing the number of
females inseminated, and (iii) by ratio tests.
        On the basis of observed matings, no differences could be detected
between 6- and 8-krad-treated males and untreated males over five
consecutive matings (Feron (1966)). Steiner et al. (1962) similarly
state that at maturity 10- to 12-krad-treated males are fully competitive.
Katiyar and Ramirez (1969) found little or no reduction in competitiveness
of 6-, 8- and 10-krad-treated males.
        However, when competitiveness is assessed on sperm transfer,
a different picture emerges. Both Katiyar and Valerio (1964a) and
Steiner et al. (1962) found that at 10 krad treated males were only about
50% effective and the latter authors further found that the amount of
sperm transferred by treated males was below normal. The competi-
tiveness, and sperm complement, increased as the time of irradia-
tion approached adult eclosion. Males irradiated at -1 day, while only
half as competitive as untreated males, were almost 6 times as com-
petitive as males treated at -3 days (Katiyar et al. (1966)).
        In laboratory ratio tests, although the degree of egg sterility is
proportional to the ratio of irradiated to untreated males (Steiner and
Christenson (1956)) the actual sterility at any given ratio is always
less than the theoretical sterility. Within the range 2 : 1 to 9 : 1 (ir-
radiated males : untreated male) the data of Katiyar and Valerio (1964a)
indicate that the competitiveness of 10-krad-treated males was about 50%

that of untreated males. To achieve an egg hatch of less than 5%, over-flooding ratios of 40 : 1 (Katiyar and Ramirez (1969)) to 64 : 1 (Feron (1966)) have been reported.

In large cage tests in Hawaii, a ratio of 10 : 1 prevented population increase, while a 20 : 1 ratio gave up to 92% suppression of the population in two generations (Steiner et al. (1962)). In a field test on caged coffee trees in Central America, Katiyar and Ramirez (1969) obtained an egg hatch (based on eggs from mature coffee barries) of 3% with an 80 : 1 ratio compared with 98% in the control over an 8-week period.

(b) Female polygamy

Whilst polygamy of females does not invalidate the sterile-male technique, this characteristic does necessitate the study of aspects of mating which are not important in the case of monogamous females. In polygamous species, not only the treated males but also their sperms must be competitive. Secondly, it should be determined whether or not the female preferentially utilizes sperms from her first or second mating. These factors, in the presence of both sterile and fertile males, assume importance when the sequence of mating is considered.

Although one mating is sufficient to ensure good egg fertility for 3 - 4 weeks (Katiyar and Ramirez (1969)), females of $\underline{C}$. capitata are polygamous, although to a limited degree. For example, in alternate mating experiments, only 15% of females mated with untreated males if they had mated 7 days earlier with a sterile male, and only 8% mated with a sterile male if they had mated earlier with a normal male (Katiyar et al. (1966)). In a further experiment the respective figures were 21% and 12% (Katiyar and Ramirez (1969)). These data suggest either that copulation with a sterile male does not satisfy a female's mating requirement as adequately as copulation with a fertile male, or that the fertile males are more aggressive. Since we have already noted that, on the basis of observed copulations, treated males did not appear inferior to untreated males perhaps there is some truth in both explanations.

The first work on alternate matings was reported by Steiner and Christenson (1956). They found that a sexually mature, sterile male required exclusive access to a female for about 10 days to effectively prevent subsequent fertilization by untreated males. Conversely, they found that the sterile male was unable to substantially reduce the fertility of a female previously mated with an untreated male. This conclusion is not supported by more recent work (Katiyar and Valerio (1964a, b), Katiyar et al. (1966), Katiyar and Ramirez (1969), which did, however, show that the change in egg hatch which occurred when a second mating with a fertile male followed a mating with a sterile male was much greater than when the reverse mating sequence was allowed. An example from Katiyar et al. (1966) will illustrate this. A fertile mating following a sterile mating changed the corrected egg hatch from 2% to 87%, while a change from 100% to 43% resulted when a sterile mating followed a fertile mating. This suggests that sperms of irradiated males are not as competitive as those from untreated males or that sterile males transfer fewer sperms.

6. DOSAGE CHARACTERISTICS

It appears that the energy of the gamma radiation used to induce sterility does not affect the degree of sterility induced by a given dosage. Results obtained with ^{137}Cs (γ energy 0.66 MeV) were essentially the same as those obtained with ^{60}Co (γ energy 1.17 + 1.33 MeV) (Katiyar (1962), Katiyar and Valerio (1964a)).

Similarly, dose rate seems to be unimportant. The data in Table I were obtained with dose rates ranging from 2.4 krad/min to 9.2 krad/min for ^{60}Co and from 75 rad/min to 1 krad/min for ^{137}Cs. The Hawaiian workers found no difference in results obtained with 7 rad/min and 1 krad/min (Steiner and Christenson (1956), Steiner et al. (1962)).

Unfortunately there are no data on the effect of fractionation of the dosage. Arroyo et al. (1965b) initiated such a study, but, apart from the fact that no egg hatch was obtained from any treatment, their trial design was such that an evaluation of the effect of fractionation would not have been possible.

7. NEUTRON IRRADIATION

The only work with neutron irradiation has been conducted by Causse et al. (1968). All irradiations were given to pupae 2 days before adult emergence. With gamma radiation, a dosage of 10 krad was necessary for almost total sterility in males but with neutrons 1.25 krad gave a sterility of 0.3%. Thus the relative biological effectiveness of neutrons is about 8 times that of gamma radiation. An R.B.E. value of about 5 is obtained when the effect on female fecundity is considered.

However, while emergence of adults was not affected by neutron irradiation of up to 1.25 krad, this dosage markedly reduced the longevity of males. The longevity of females was only slightly reduced.

8. CHEMOSTERILIZATION

Keiser et al. (1965) administered tepa, apholate, metepa and tretamine to pupae and adults of C. capitata in a variety of ways. All chemicals successfully sterilized both sexes, although higher concentrations were required for females than males. This sexual difference in susceptibility has been noted by Orphanidis (1963) and Orphanidis et al. (1963), who also found that apholate, metepa and tepa, administered orally, gave good sterility which persisted for at least 14 days. The only other data found on chemosterilization was from Germany where metepa was found effective (Scherney and Haisch (1968)).

9. CONCLUSIONS

In this paper I have carefully avoided ascribing the sterility produced in the males to dominant lethal mutations. Whilst it is probably true that dominant lethal mutations are induced in the gametes, no cytological evidence has been produced to prove this point. I think

there is an indication, from the alternate mating experiments, that a degree of sperm inactivation may be involved, and certainly aspermia becomes an important factor as the irradiated males age. In females the most important cause of sterility is infecundity.

It would appear that for the current field suppression experiments with C. capitata, the criterion for the selection of the pupal irradiation dosage has been primarily, if not solely, based on the degree of sterility produced in males. This has been assessed from crosses between irradiated males and untreated females. Overflooding ratios then have been increased to compensate for the lack of competitiveness of the treated males. Recent work, by ourselves and by Dr. Katiyar (see the paper in these Proceedings by Hooper and Nadel and the short contribution by Katiyar and Ramirez), suggests that we may be better off adopting a lower dosage than currently used. Whilst this lower dosage will give a somewhat lower level of sterility, in the long term this may be compensated by increased competitiveness of the males with a consequent reduction on the overflooding ratio.

It is in the area of competitiveness and mating behaviour of the male that I think more work is needed. Firstly, in the light of the comments above, reasonably large-scale experiments to test the interaction between irradiation dosage and competitiveness should yield useful information. Secondly, depending on whether the act of copulation or sperm transfer has been used as the criterion, it has been noted above that different evaluations of competitiveness have been obtained. More experimental work is needed to reconcile these two evaluations. Finally, there seems to be a dearth of information on how often, and how frequently, a male will mate.

REFERENCES

ARROYO, M., JIMENEZ, A., MELLADO, L., CABALLERO, F. (1965a). Aplicación de isótopos radiactivos a la investigación de metodos sobre lucha biologica contra las plagas. III. Obtención de "Machos esteriles" de Ceratitis capitata Wied., mediante la irradiación de sus pupas con rayos gamma, Trabajos (Series Fitopatología) num.390, Estación de Fitopatología Agricola de Madrid.

ARROYO, M., JIMENEZ, A., MELLADO, L., CABALLERO, F. (1965b). Ibid., IV. Efectos de la radiación gamma sobre Ceratitis capitata Wied., en función del fraccionamiento de la dosis de irradiación, Trabajos (Serie Fitopatología) num.391, Estación de Fitopatología Agricola de Madrid.

CAUSSE, R., FERON, M., PEREAU-LEROY, P. (1968). "Effets comparés d'irradiations aux rayons gamma et aux neutrons sur les organes reproducteurs de la mouche méditerranéenne des fruits Ceratitis capitata Wied. (Diptera: Trypetidae)", Isotopes and Radiation in Entomology, (Proc. Symp. Vienna, 1967) IAEA, pp.355-364.

FERON, M. (1966). Stérilisation de la mouche méditerranéenne des fruits, Ceratitis capitata Wied., par irradiation des pupes aux rayons gamma, Annls Epiphyt. 17, 229-239.

FERON, M., SERMENT, M.-M. (1963). Différenciation sexuelle aux stades œuf et pupe chez la mouche méditerranéenne des fruits Ceratitis capitata Wied., Revue Path. vég. Ent. agric. Fr. 42, pp.239-244.

KATIYAR, K.P. (1962). "Possibilities of eradication of the Mediterranean fruit fly, Ceratitis capitata Wied., from Central America by gamma-irradiated males", Fourth Inter-American Symposium on the Peaceful Applications of Nuclear Energy (Mexico City, 9-13 April, 1962) pp.211-217.

KATIYAR, K.P., RAMIREZ, E. (1969). "Sterilization of the Mediterranean fruit fly and its application to fly eradication", in The Application of Nuclear Energy to Agriculture, Triennial Report, July 66 - 30 June 1969, for contract AT (30-1)-2043 of Inter-American Institute of Agricultural Science of the OAS, Turrialba, Costa Rica, pp.90-105.

KATIYAR, K.P., VALERIO, J. (1964a). "Further studies on the possible use of sterile-male release technique in controlling or eradicating the Mediterranean fruit fly, Ceratitis capitata Wied., from Central America", Fifth Inter-American Symposium on the Peaceful Applications of Nuclear Energy, Valparaiso (9-12 March, 1964) pp.197-202.

KATIYAR, K.P., VALERIO, J. (1964b). "Sterilization of the Mediterranean fruit fly and its application to fly eradication", Annual Report, 1 July 1964, of the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica to U.S. Atomic Energy Commission, pp.51-67.

KATIYAR, K.P., FERRER, F., VALERIO, J. (1966). In: The Application of Nuclear Energy to Agriculture, Triennial Report, 1 July 1963 - 30 June 1966, of the Inter-American Institute of Agricultural Sciences of the OAS, Turrialba, Costa Rica, pp.45-60.

KEISER, I., SCHNEIDER, Esther L. (1969a). Need for immediate sugar and ability to withstand thirst by newly emerged Oriental fruit flies, melon flies, and Mediterranean fruit flies untreated or sexually sterilized with gamma radiation, J. econ. Ent. 62, pp.539-540.

KEISER, I., SCHNEIDER, Esther L. (1969b). Longevity, resistance to deprivation of food and water, and susceptibility to malathion and DDT of Oriental fruit flies, melon flies, and Mediterranean fruit flies sexually sterilized with tepa or radiation, J. econ. Ent. 62, pp.663-666.

KEISER, I., STEINER, L.F., KAMASAKI, H. (1965). Effect of chemosterilants against the oriental fruit fly, melon fly and Mediterranean fruit fly, J. econ. Ent. 58, pp.682-685.

LINDQUIST, A.W. (Ed.)(1963). Insect Population Control by the Sterile-Male Technique, (Report Panel Vienna, 1962) IAEA, Tech. Rep. Ser. No.21, pp.28-29.

ORPHANIDIS, P.S. (1963). Recherches en laboratoire sur la stérilisation d'adultes de Ceratitis capitata Wied. au moyen de substances stérilisantes. I. Expériences avec l'apholate, Annls Inst. phytopath. Benaki 5, pp.260-287.

ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., KARANDEINOS, M.G. (1963). Recherches en laboratoire sur la stérilisation d'adultes de Ceratitis capitata Wied. au moyen de substances stérilisantes. II. Expériences au moyen de metaphoxide, d'aphoxide et de chlorine de cuivre, Annls Inst. phytopath. Benaki 5, pp.323-331.

SCHERNEY, von F., HAISCH, A. (1968). Zur Zucht und Sterilisation von Schadinsekten, insbesondere der Mittelmeerfruchtfliege Ceratitis capitata Wied., Anz. Schädlingsk. 41, pp.7-14.

SHOUKRY, A. (1968). Studies on the ecology of the Mediterranean fruit fly C. capitata in U.A.R. with special reference to the biological effects of gamma radiation. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Summarized in: "Studies on the ecological factors affecting the control of the Mediterranean fruit fly, Ceratitis capitata Wied. in U.A.R. by the use of the sterile male technique", Final Report on the IAEA Research Contract 408/RI/RB for period 1966-1968, IAEA, Vienna.

STEINER, L.F., CHRISTENSON, L.D. (1956). Potential usefulness of the sterile fly release method in fruit fly eradication programmes, Proc. Hawaii. Acad. Sci. 3, pp.17-18.

STEINER, L.F., MITCHELL, W.C., BAUMHOVER, A.H. (1962). Progress of fruit fly control by irradiation sterilization in Hawaii and the Marianas Islands, Int. J. appl. Radiat. Isotopes 13, pp.427-434.

CURRENT MASS-REARING TECHNIQUES FOR THE MEDITERRANEAN FRUIT FLY

D. J. NADEL
International Atomic Energy Agency,
Vienna

Abstract

CURRENT MASS-REARING TECHNIQUES FOR THE MEDITERRANEAN FRUIT FLY.
Techniques employed by a number of laboratories which are mass-rearing Ceratitis capitata
(Wiedemann) are reviewed. The aspects covered include egg production, larval media, collection of
larvae and pupae, and handling of pupae.

INTRODUCTION

In the past decade the Mediterranean fruit fly (medfly), Ceratitis
capitata (Wiedemann), has gained world-wide recognition as a valuable
research insect in entomology. This recognition can be attributed to the
relative ease with which this highly adaptable insect can now be mass-
produced. The stimulus for the mass production of medfly came from the
need for large numbers of experimental flies for sterile-insect release
programs, for laboratory hosts for mass rearing of larval parasites of the
olive fly, Dacus oleae (Gmelin), and for laboratory research programs which
require large numbers of insects (as in development of fly attractants).

With the medfly's trait of extreme adaptability to insectary conditions
and taking into account the ever-increasing numbers of laboratories involved
in medfly research and production, it would be difficult, if not impossible,
to report on all rearing techniques now employed.

The purpose of this report is to summarize and compare rearing
methods in the following insectaries where production of over one million
pupae per day has been reached:

 (1) USDA Fruit Fly Laboratory, Honolulu, Hawaii
 (2) OIRSA, UN Special Fund, IAEA, San José, Costa Rica
 (3) Institute of Agricultural Entomology, University of Palermo, Sicily
 (4) IAEA Entomology Laboratory, Seibersdorf, Austria.

LARVAL DIET

Table I indicates the adaptability of medfly larvae to diets of differing
textures and ingredients. Sodium benzoate, Nipagin, sugar, brewer's yeast
(or torula yeast), HCl and tap water are common to all the diets (with the
one exception that Nipagin is omitted from the Palermo diet).

Diet texture, bulk and additional nourishment are provided by the
addition of wheat shorts, Gelgard M and middlings in the Hawaiian diets;
wheat germ and bagasse in the San José diet; wheat bran in the Seibersdorf
diet; and lucerne grass powder plus crushed straw in the Palermo diet.
The inclusion of the latter four ingredients is a good example of adaptation
of rearing methods to local conditions, resulting in more economic
production.

TABLE 1. LARVAL REARING DIETS FOR MEDITERRANEAN FRUIT FLY
(Approx. % by weight)

Ingredients	(U.S.D.A.) HAWAII		(O.I.R.S.A.) SAN JOSÉ Popping diet	(I.A.E.A) SEIBERSDORF Popping diet	(Univ. of Palermo) SICILY Popping diet
	Washing diet	Popping diet			
Sodium benzoate	0.1	0.1	0.1	0.1	0.2
Nipagin (methyl-para-hydroxy benzoate)	0.1	0.1	0.1	0.1	-
Granulated sugar	12.4	12.4	18.0	18.0	8.0
Dry brewer's type yeast	3.1	3.1	7.8	8.5	5.6
HCl (conc.)	0.9	0.9	0.7	2.3	2.0
Tap water	59.8	64.8	63.5	50.0	66.3
Wheat shorts	5.0	17.8	-	-	-
Gelgard M	0.8	0.8	-	-	-
Middlings	17.8	-	-	-	-
Wheat germ	-	-	7.1	-	-
Bagasse	-	-	7.7	-	-
Wheat bran	-	-	-	26.0	-
Lucerne grass powder (Medicago sativa L.)	-	-	-	-	9.4
Crushed straw (Triticum durum L.)	-	-	-	-	8.5
Number of ingredients	9	8	8	7	7

OVIPOSITION CAGES

Medfly oviposition cages used in mass-rearing programs range from 60 × 60 × 80 cm (Palermo) to 200 × 140 × 40 cm (San José). With the exception of the new Seibersdorf cage which is cylindrical, all the cages are of rectangular shape.

Oviposition sites provided are of two basic types: the perforated plastic tube (Hawaii) or the perforated plastic tunnel (Palermo), and the synthetic cloth screen (San José and Seibersdorf). The collection of eggs from trays of water set beneath the oviposition unit where synthetic cloth is utilized is less time-consuming and more readily adaptable to automation than collection from the perforated-tube site. However, where oviposition is through the synthetic cloth, eggs tend to stick to the walls of the cage as the resting flies dirty the cloth material.

Water is usually provided by agar cakes consisting of 0. 75 to 1. 0% in water. Food usually consists of a mixture of 1 part yeast hydrolysate (i. e. N. B. C. or acceptable substitute) to 3 - 5 parts sugar. In Hawaii the yeast is presented separately from the sugar.

The cylindrical cloth cage (Fig. 1) developed at Seibersdorf permits the fullest use of the room height, and the 70% terylene : 30% cotton material, with its smooth finish and 16 mesh/cm, permits good aeration and visibility. The flies lay eggs through the total height and circumference of the cloth cage sides.

EGG COLLECTING AND HANDLING

Eggs are collected once daily and usually seeded on the larval diet after volumetric measurement (estimates run from 20 000 - 25 000 eggs per ml). The USDA Laboratory in Hawaii has developed an accurate and efficient method of egg seeding, whereby the eggs are held with some water in catsup dispensers and apportioned into small plastic tubes closed on the lower end by squares of organdy cloth. The cloths and plastic tubes are held upright by insertion into appropriately sized holes drilled in a board. After the water has drained through the organdy cloth slight adjustment is made to the level of the eggs in the tube to ensure equal egg distribution on the medium. By lifting the plastic tube the eggs fall onto the organdy cloth which is then inverted and the eggs are smeared on the larval diet.

COLLECTION OF MATURE LARVAE

In Hawaii, mature larvae are collected by two methods (see also Table I): "washing" and "popping". In the washing method initial popping of mature larvae begins and these larvae are collected in moist vermiculite. The remaining larvae are collected by washing the diet through an appropriately sized sieve. The larvae thus collected are transferred to a cement mixer containing moist vermiculite and mixed for 5 minutes.

All the other laboratories use the "pop" method exclusively. In this technique the mature larvae leave the medium by crawling and popping off the sides of the stacked larval medium trays and fall into collecting boxes set below the rearing unit. An interesting adaptation of the method was
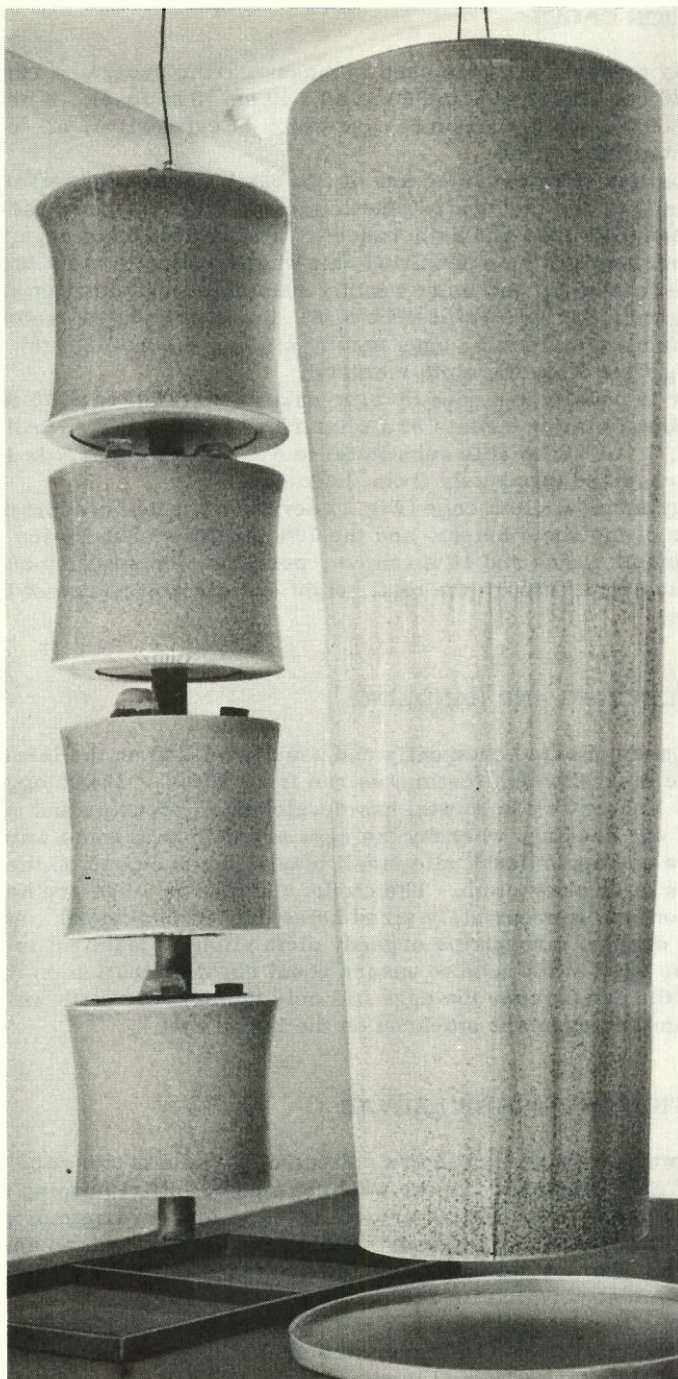
FIG. 1. Cylindrical oviposition cages at Seibersdorf.

developed in San José where the trays with maturing larvae are inverted onto hardware screening and the larvae fall from the medium onto guide boards, which direct them to the collecting box below.

It would seem that the simplest and most convenient collection method is simply to allow the maturing larvae to leave the tray and fall into collecting boxes, as practised at Seibersdorf, provided that high humidity can be maintained in the rearing cabinets or room (larvae will pupate in dried-out medium in the larval rearing tray). Sawdust (San José), granular river sand (Sicily) and fine bran (Seibersdorf) are used as pupation media; these materials are presented dry to the popping larvae.

PUPAL HANDLING

In Hawaii pupae are held in the moistened vermiculite for at least 4 days. Separation is by means of a continuous-flow sifter and the naked pupae are spread about 1 cm deep in screen-bottomed trays. At Seibersdorf pupae have been separated from the bran medium as early as 24 hours after collection without affecting eclosion. However, separation is usually on the 3rd or 4th day after collection.

ROOM ENVIRONMENT

Conditions in the rooms should be as follows:

(1) Oviposition room

    (a) Temperature: 25 - 27°C is considered satisfactory.

    (b) Relative humidity: Extremes of relative humidity can be harmful to the adults, especially in regard to food availability. Over-dry conditions may make the yeast-sucrose combination too hard and crusty. Conversely, high humidity may turn the food into a sticky trap for the feeding flies.

(2) Larval rearing room

    (a) Temperature: In Hawaii cultures of both the washing and popping diets are held at 27 ± 2°C for 5 days after seeding and then transferred to 20 ± 2°C for the remaining 2 - 4 days to prevent overheating in the wooden rearing cabinets. At Seibersdorf we have discontinued using closed cabinets in favour of an open rearing system set on a continuous overhead rail. With room temperatures held at 25 - 27°C trays do not overheat.

    (b) Relative humidity: In closed cabinets high relative humidities ensure good larval hatch even when humidity in the rearing room is low. However, unless facilities are available for cooling the cabinets at the end of the production cycle, overheating accompanied by premature popping and high larval and/or pupal mortality may result. In open rearing systems (as practised at Seibersdorf) room humidity must remain high (around 80%) to avoid egg desiccation and drying out of the larval medium. One solution to

ensure proper conditions for the hatching larvae is to enclose
the freshly loaded rearing unit in plastic sheeting for 3 days until
hatch is complete.

(c) Lighting: Continuous lighting is used in oviposition rooms
except in San José, where a 16-hour light period is used. Where
rectangular cages are employed it is especially important that
light intensities be equal on all screened surfaces, to avoid
"pile-up" and loss of the adult fly stock. This problem is
avoided with the cylindrical cloth cage. The flies predominate
where light intensity is greatest but there is no critical pile-up.

Exclusion of Drosophila spp.

Drosophila spp. are notorious pests in medfly cultures. The best
control seems to be to take extreme care to exclude Drosophila from the
larval rearing units while loading is underway. We were able to reduce and
control a critical infestation of Drosophila in the summer of 1969 by
designing and fitting a 100% leak-proof netting on each larval rearing unit.
Before unit loading, the walls and ceiling and the unit itself were sprayed
with water. This forced those Drosophila not drowned to find dry resting
areas some distance from the unit. After loading and sealing the unit, indi-
vidual Drosophila that appeared were killed as they rested on the inside of
the netting.

FACTORS LIMITING EFFICIENT PRODUCTION

The following points indicate some factors that limit efficient production:

Methods and materials used with success and economy in one region
may not be applicable elsewhere because of supply limitations or
high cost.

Production set-ups in 'temporary' buildings not designed for insectary
purposes can be very inefficient.

Budgetary limitations, the pressure to adapt existing materials, such
as oviposition cages, to mass rearing, are self-defeating and limit
the development of highly efficient and economical production.

Quality control of food supplies should be run before supplies of proven
materials run short.

METHODS OF MASS REARING
THE MEDITERRANEAN FRUIT FLY
CURRENTLY USED BY THE
U. S. DEPARTMENT OF AGRICULTURE

N. TANAKA, R. OKAMOTO, D. L. CHAMBERS
Entomology Research Division,
Agricultural Research Service,
United States Department of Agriculture,
Honolulu, Hawaii,
United States of America

Abstract

METHODS OF MASS REARING THE MEDITERRANEAN FRUIT FLY CURRENTLY USED BY THE U. S. DEPARTMENT OF AGRICULTURE.

Recent improvements in the methods of rearing the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (including equipment, techniques, and diets) have resulted in lower costs and easier handling. Some specific changes are: the substitution of milled wheat for dehydrated carrot powder; the addition of a water-binding agent to the larval diet; and the development of semiautomated methods of collecting larvae and pupae.

Mitchell et al.[1] (1965) described a low-cost method of rearing the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), that was then in use by the U.S. Department of Agriculture. However, since 1965, researchers have been able to make several modifications and improvements to this method including the development of a larval diet containing milled wheat instead of dehydrated powdered carrot. This report describes the current rearing methods used by the U.S. Department of Agriculture and the changes that have been made since 1965. Each phase of rearing is discussed separately.

ADULTS

About 30 000 adult flies are housed in each 30.5 × 61.0 × 121.0-cm cage made of 0.95-cm plywood with 16-mesh per 2.54-cm screening at the top and upper 17.8 cm of each side. The five circular openings on each side of the cage receive the egg receptacles when they are inserted. Cages are stacked 4 high on a movable base with 10.2 cm between each cage.

The adults are held at a temperature of 27.0 ± 2°C and a relative humidity of 50-70% for 3 weeks after eclosion. Light is provided by 8-ft, 80-W fluorescent lights affixed to an 8-ft-high ceiling above the cages.

---

[1] MITCHELL, S., TANAKA, N., STEINER, L. F. (1965). Methods of mass culturing melon flies and oriental and Mediterranean fruit flies, U. S. Dept. of Agriculture ARS 33-104, 22 pp.

The components of the adult diet, 453 g of granulated sugar and
226 g of enzymatic yeast hydrolysate (Nutritional Biochemical Corp.), are
placed separately on the cage floor. Water is provided by pouring 0.75%
agar in water (by weight), a development of A. Peleg in Costa Rica, into
7.6 × 30.5-cm pleated polyethylene bags, allowing it to solidify, and
offering it to the flies from the outside top of the cage after one side of the
bag has been cut open. Two bags each containing 600 ml of agar-water
are placed on each cage each week. The enzymatic yeast hydrolysate (NBC)
which costs 2 US dollars per pound is a temporary replacement for the
cheaper Type M (R) (Standard Brands, Inc.) which is no longer manufactured.
However, tests now underway indicate that Amber BHY Series 100 (R)
(Amber Laboratories) which costs 61 US cents per pound is comparable to
the NBC yeast hydrolysate.

EGG COLLECTING

Eggs are collected three or more times per week starting 4 days after
adult emergence. A 2.5 × 2.5 × 5-cm cellulose sponge is placed in a
container perforated with about 300 holes of 0.3 mm diameter. The sponge
is saturated with tap water to provide the ovipositional stimulus and to
prevent the eggs from desiccating. Eggs are collected by inserting the
container into the openings at the sides of the cages and leaving them
there for 18-20 hours. Then the containers are removed, and the eggs
are washed under running water into a net from which they are transferred
to a polyethylene squeeze-bottle.

EGG CALIBRATION

Tubes made from 3-dram plastic vials (cut off at the bottom) that
will hold a volume of 6 ml (150 000 eggs) and a 30.5 × 61.0 × 0.95-cm
board containing 32 2.5-cm-diam. holes are used to apportion the eggs
held in the squeeze-bottle. First, a 7.6-cm organdy square is placed
under each tube, and the cloth and tube are inserted into the holes on the
board; then the tubes are filled with eggs. The water drains through the
organdy, the eggs are levelled, and any excess eggs are removed with
a camel's-hair brush. The eggs are transferred to the organdy square
by lifting the tube.
Unhatched eggs are seeded onto larval diet soon after they are
apportioned. Periodic checks on egg hatch are made by placing samples
on a moist blotter held in a Petri dish and recording the number hatched.

LARVAL DIET

Two diets are available. One is used when the larvae are to be re-
covered by washing them from the spent medium in a sieve under running
water (washing method). The second is used when larvae are to be
recovered by allowing them to leave the diet ad libitum (popping method).
In the first diet, wheat middlings (also referred to as 'red dog') and
wheat shorts are used at a ratio of about 4:1 to permit rapid passage of

TABLE I. COMPOSITION OF THE TWO DIETS

Ingredients	Washing diet (% by weight)	Popping diet (% by weight)
Sodium benzoate	0.1	0.1
Nipagin ® (methyl-para-hydroxybenzoate)	0.1	0.1
Granulated sugar	12.4	12.4
Dried torula yeast (Type 200)	3.1	3.1
HCl (concentrated)	0.9	0.9
Wheat shorts	5.0	17.8
Gelgard M ® (moisture control agent)	0.8	0.8
Middlings	17.8	None
Tap water	59.8	64.8

Note: The prepared diets should have a pH of 4.5.

the spent diet through the sieve when the larvae are washed out. The shorts give the diet a texture that is manageable when it is dispensed into the rearing trays. The second diet, which has no middlings, is wetter than the first. The formulas for the two diets are given in Table I.

MIXING PROCEDURE

The mixing procedure is as follows:

(1) With a 127-litre capacity concrete mixer, cold-mix water, preservatives, granulated sugar, and torula yeast for about 5 min.
(2) Add HCl and mix thoroughly.
(3) For the washing diet, blend together Gelgard M and shorts, add mixture to above, and mix until clumps disappear. For the popping diet, blend Gelgard M with one half of the required shorts.
(4) For the washing diet, add middlings and mix until smooth consistency is attained. For the popping diet, do the same with the remaining shorts.

WASHING METHOD OF RECOVERING LARVAE

Twenty rearing trays are stacked in each half of a 182.9 × 91.4 × 91.4-cm wooden cabinet that is partitioned at the centre with fine lumite screening and has doors on two sides. The larvae mature 6 days after the eggs are seeded and begin to leave the diet (pop). Popped larvae (usually 20 to 40% of the total) drop to the bottom of the cabinet which contains 18 litres of moist (5 to 20% water) vermiculite; the mixture of larvae and vermiculite is transferred to holding boxes.

The trays containing the larvae that have remained in the diet are emptied into a 200-litre vat, 10 trays at a time, and contents of the vat are diluted with water. The resultant slurry drains into 16-mesh per 2.54-cm sieves through a 3.81-cm valve at the bottom of the vat and is washed in the sieves under running water. Washed larvae are placed in a bucket (held with some water until the bucket is filled); then they are poured into a cloth bag so that the excess water can drain off, and transferred to a cement mixer containing moist (5 to 20% water) vermiculite at a ratio of 1 litre of larvae to 6.0 litres of vermiculite. After the concrete mixer has been operated for about 5 min, the mixture is transferred to a hopper which has an opening at the bottom that is fitted with a denim sleeve. The hopper is raised on a hoist, and 7.0 litres each of the mixture is dispensed through the sleeve into 15.2 × 30.5 × 45.7-cm fibreglass stackable boxes. As many as 2.0 million larvae are recovered from 20 trays.

POPPING METHOD OF RECOVERING LARVAE

A 391.2 × 71.2 × 15.2-cm galvanized pan with a 2.8-cm drain at the corner of one end is placed on a 365.8 × 61.0 × 45.7-cm wooden platform with casters which has a 2.5-cm inclination lengthwise. The drain rests on the low end of the platform. Then four stacks of 25 trays each are placed lengthwise on a 320.0 × 35.6 × 17.8-cm stand of 1.9-cm galvanized pipe that is inclined 2.5 cm with the low end opposite that of the platform. A 381.0 × 61.0 × 180.0-cm lumite screen enclosure on a wooden frame with 7.6-cm legs of 0.3 × 2.5-cm aluminium angle iron rests inside the galvanized pan. One day before the larvae begin to pop, the galvanized pan is filled with water to a depth of 2.5 cm. The popping larvae drop into the water and are drained into a cloth bag and mixed with vermiculite. The subsequent procedure is similar to that described for the washing method. Larvae are recovered from the water twice per day. (No adverse effects have been noted in larvae held as much as 24 hours in water.) About 90% of all recovered larvae pop in 3 days, and about 5% pupate within the diet.

Cultures in both diets are held at a temperature of 27 ± 2°C for 5 days after egg set and are then transferred to a temperature of 20 ± 2°C for 2 days (washing diet) or 4 days (popping diet) to prevent overheating.

PUPAL HANDLING

Pupae are sifted not earlier than 4 days after pupation in a 30.5-cm-diam. × 243-cm open-end, continuous-flow sifter made of 16-mesh per 2.5-cm screen. The sifter is mounted to a $\frac{1}{2}$ h.p. motor and rotates at 18 rev/min. Two litres of the sifted pupae are each placed in 50.8 × 50.8 × 2.5-cm (inside dimensions) screen-bottomed trays and spread about 1 cm deep.

The trays are stored and kept in stacks (separated by 5.1-cm cleats). If the pupae are now exposed to a temperature of 20°C, the development is slowed to half the rate for a temperature of 27°C. Therefore, pupae of varying ages can be produced by manipulating the temperature.

SANITATION

Adult cages, larval cabinets, and rearing trays are washed in hot water (82°C).

PRODUCTION COST

Material cost per million pupae is estimated as 10 US dollars. This amount includes the cost of the protein fed to the adults, the cost of the ingredients for the larval diet, and the cost of other expendable items.

SPACE AND PERSONNEL REQUIREMENTS

It is estimated that six to eight experienced workers in a properly equipped building of about 270 m^2 could rear 60 million Mediterranean fruit flies per week by using these techniques.

Note

Mention of a proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.

PHYSIOLOGY OF THE MEDITERRANEAN FRUIT FLY IN RELATION TO THE STERILE-MALE TECHNIQUE

P. A. LANGLEY*
International Atomic Energy Agency,
Vienna

Abstract

PHYSIOLOGY OF THE MEDITERRANEAN FRUIT FLY IN RELATION TO THE STERILE-MALE TECHNIQUE.

The results of several workers indicate that the environment can affect the composition or extent of nutritional reserves in the pupae of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). However, there is no information in the literature on this subject. This is unfortunate since the nutritional status of the mature 3rd instar larva determines the survival potential of the insect during metamorphosis, and until the emergent adult can obtain and digest its own food.

We have shown that the temperatures to which the pupae are subjected affect the extent of fat reserves in the emergent adults. Incubation above 25°C for the whole of pupae life lowers these reserves significantly. Measurements of respiratory quotient indicate that fat is the main energy reserve during pupal development and the total amount of oxygen consumed is in reasonable agreement with this when compared to the total amount of fat consumed. Similar measurements on newly emerged, unfed adults, indicate that both carbohydrate and fat are used as energy sources.

Thus any treatment which tends to accelerate the consumption of reserves in the pupa without a corresponding acceleration in developmental rate must affect the survival potential of emergent adults. A study of the physiological relationships of the Mediterranean fruit fly to its environment in quantitative terms is essential to the correct interpretation of much of the highly successful empirical work on mass rearing and the sterile-male technique as applied to this insect.

INTRODUCTION

In the Preface to the first edition of "The Principles of Insect Physiology" in 1939, V.B. Wigglesworth wrote: "The physiology of insects is to some the handmaid of economic entomology .... The rational application of control measures .... is often dependent on a knowledge of the physiology of the insect in question. Physiology may thus serve to rationalize existing procedures, or to discover the weak spots in the ecological armour of a species. A knowledge of the ecology of a species is always necessary to its effective control; its ecology can be properly understood only when its physiology is known."

Insect physiology can only be studied if a regular supply of research material is available. This poses no problem when an insect is mass-reared for the application of the sterile-male technique. However, the steps involved in solving physiological problems are not always meaningful to authorities financing research which is directed at insect pest control. Thus it is not surprising to find that most of our knowledge of the physiology of fruit flies relates only to Drosophila species, which are easily reared in sufficient numbers to supply the needs of the physiologist, in universities and other academic institutions.

---

* Present address: Tsetse Research Laboratory, Dept. of Veterinary Medicine, University of Bristol, Langford House, Langford, Bristol, United Kingdom.

The development of artificial diets and mass-rearing techniques for the Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), has been almost entirely empirical and highly successful. The definition of optimal rearing conditions has been based on the number of viable pupae obtained for a given number of eggs. It is my purpose to show how a knowledge of medfly physiology will influence decisions on optimal rearing conditions, and how it may alter the definition of 'quality' as applied to emergent adults.

The effect of temperature upon developmental processes is not always the same as that upon utilization of energy reserves. Hence there is often a temperature at which development proceeds with the minimum expenditure of energy (Keister and Buck (1964)). This temperature is not necessarily the optimum as far as survival or speed of development is concerned. It is the temperature of greatest 'developmental efficiency'. Neither is it always a constant temperature; fluctuating temperatures about a mean are superior in some insects (Keister and Buck (1964)).

The effect of humidity upon developmental processes will depend on the ability of the insect to regulate its water balance (Bursell (1964)), while light will influence development in those insects where diurnal rhythms are known to exist.

THE LARVA

Under natural conditions the medfly larva is not affected by light or humidity while it is enclosed in its food medium. Temperature, however, is important.

The purpose of the larval stage is to provide the necessary nutritional reserves, for metamorphosis within the puparium, and to provide energy for the emergent fly until it can find food of its own.

The gain in developmental rate from 27°C to 32°C is equal to that from 24°C to 27°C; one or more stages develop most rapidly at about 32°C, and temperatures above 32°C are harmful (Mitchell, Tanaka and Steiner, 1965). If the temperature of the larval medium rises too high, larvae tend to leave it prematurely, with consequent formation of small pupae and increased mortality (Nadel, personal communication). However, Myburgh (1963) states that high temperatures (30°C) inhibit emergence of larvae from infested fruit (in this case peaches), and there can be a delay of 2 or 3 weeks in the development of the 3rd instar. Mourikis (1965) states that pupal duration is proportional to larval duration, depending upon which fruit the larva developed in. A similar phenomenon in the olive fly Dacus oleae (Gmelin) was reported by Sacantanis (1954). The relationship is apparently independent of temperature, and must therefore depend on modifications in the composition of energy reserves laid down by the larva, such that they are more or less readily utilizable by the pupa. Table I gives some examples. We cannot, however, rule out the possibility of temperatures within different fruits varying considerably according to their state of ripeness, and thus affecting the length of larval life directly.

In view of this it is surprising that there is no information in the literature on the extent and nature of energy reserves stored by mature 3rd instar medfly larvae reared at different temperatures and in different media. Nothing has been published on the relative amounts of fat and glycogen normally found in medfly larvae, and these two substances constitute the main energy stores for most insects (Wigglesworth, 1965).

TABLE I. DURATION OF LARVAL AND PUPAL PERIODS OF
THE MEDFLY REARED IN DIFFERENT HOST FRUITS AT A
TEMPERATURE OF 26-28°C (AFTER MOURIKIS (1965))

Host fruit	Duration in days	
	From oviposition to pupation	From pupation to adult emergence
Peach	7.8	9
Tomato	8	9
Pepper	8	9
Pear	12.4	11
Apple	16.5	10.5
Apricot	10	11

THE PUPA

It is well known that humidity and temperature influence the rate of
development and survival of the insect within the puparium, but again
most studies on medfly have involved the empirical approach. Environ-
mental conditions which are optimal for survival and speed of develop-
ment are generally chosen for mass-rearing programs. However, syn-
chronization of pupal development is practical where large numbers of
pupae must be irradiated on one day for release as adults soon after-
wards. This is achieved by holding pupae for varying times at tempera-
tures between 20°C and 27°C (Mitchell, Tanaka and Steiner 1965). Oc-
casionally temperatures ranging from 15°C to 28°C have to be used (Nadel,
personal communication).

We have found that by subjecting medfly pupae to different temperature
regimes involving 20°C, 25°C, and 30°C, for varying lengths of time during
their development, the duration of the pupal period is related to the inte-
grated mean temperature as shown in Fig.1. We have also found that the
fat content of flies emerging from these pupae is affected by the tempera-
tures at which they were incubated (Fig.2). From this figure it is clear
that incubation at temperatures above 25°C lowers the fat content of the
emergent flies.

The significance of fat as an energy reserve for the pupa is shown
in Fig.3, from which it can be seen that the respiratory quotient is close
to 0.7, indicating the combustion of fat reserves, throughout pupal develop-
ment. At the same time the oxygen consumption follows the typical U-shaped
curve associated with pupal respiration in insects, and it can be calculated
that a total of 110 mm^3/mg is consumed.

During uninterrupted pupal development at 25°C and 75% r.h. the fat
content falls from approximately 40% to 15% of the non-fatty dry weight
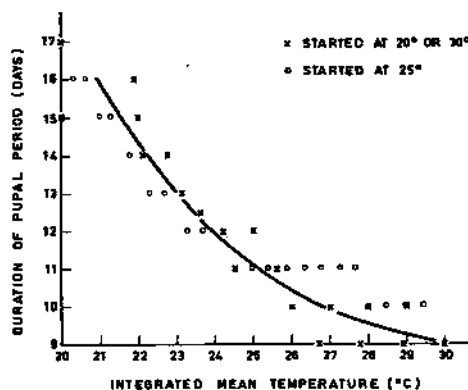
FIG. 1.   Relationship between temperature and pupal period in the medfly.
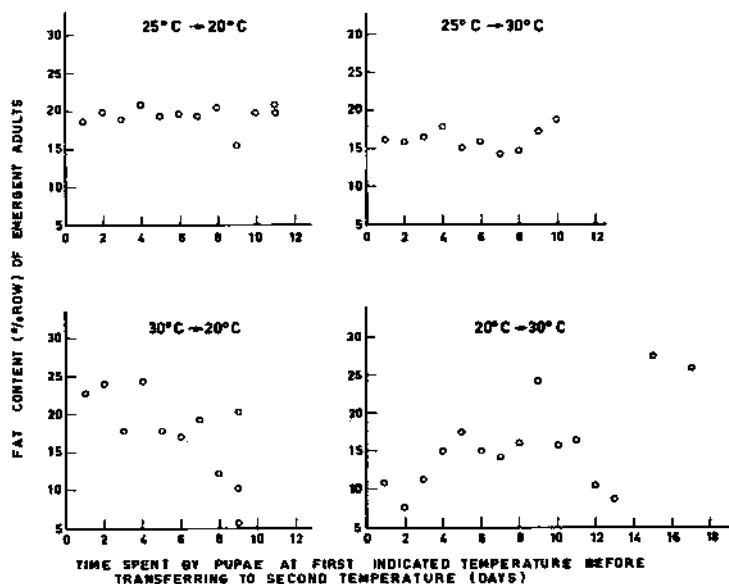
FIG. 2.   Influence of time spent at different temperatures on the fat content of emergent adults.

(Fig. 4).   The non-fatty dry weight or residual dry weight (RDW) is quite
constant and represents about 25% of the initial live weight at the time of
pupation.   Thus a pupa weighing 10 mg initially has an RDW of about 2.5 mg.
Its initial fat reserves will be about 1 mg and these will fall to 0.4 mg at
emergence.   The consumption of 0.6 mg fat corresponds therefore to an
oxygen consumption of around 1100 mm^3.   This rough calculation provides
an estimate of 1850 mm^3 of oxygen for the combustion of 1 mg of fat, which
is the right order of magnitude for fat combustion (Bursell (1959)), although
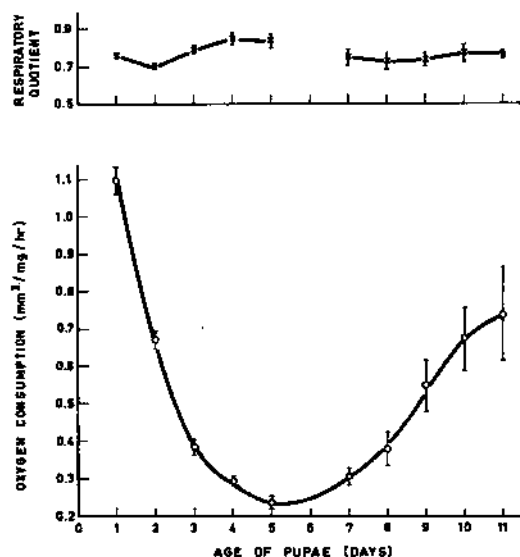
FIG. 3. R. Q. and O₂ consumption in medfly puparia at 25°C.
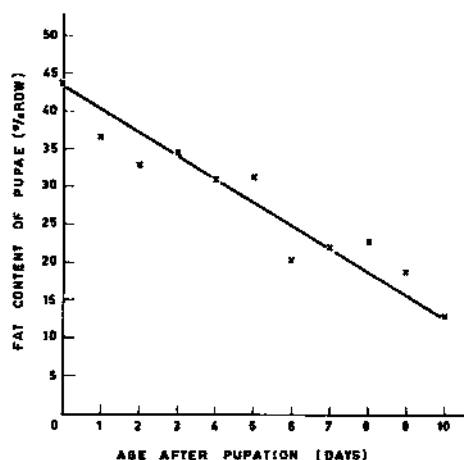
FIG 4. Influence of age of pupae on fat content.

nothing is known of the composition of medfly fat and wide variations do occur in insects (Gilmour (1961)).

As confirmation of our results on fat determinations of adults emerging from pupae held at different temperatures, the oxygen consumption of pupae held at different temperatures ought to be determined. The temperature of greatest 'developmental efficiency' would correspond to the lowest total oxygen consumption and would probably be found to be a little lower than 25°C. This temperature has been determined for Drosophila species and is 25°C (see Keister and Buck (1964)).

THE ADULT

At emergence the adult Drosophila melanogaster contains 'larval fat body'; the 'imaginal fat body' is not extensive, and there is very little glycogen present (Wigglesworth (1949)). During the next 48 hours the larval fat body diminishes, and disappears by the 4th day. With normal access to food, the glycogen store increases along with the adult fat body. The energy source for flight is glycogen while fat is not consumed during flight; after flight exhaustion, recovery is more rapid when the flies are fed on glucose than on sucrose or fructose; during starvation Drosophila consumes fat concurrently with carbohydrate (Wigglesworth (1949)). During flight the respiratory quotient (R.Q.) for Drosophila is 1.0, indicating the combustion of carbohydrate (Chadwick (1947)).

No similar information is available in the literature on C. capitata. Our own studies have shown that during the 3 hours after emergence the R.Q. of the adult medfly is between 0.9 and 1.0. This suggests a rapid switch from fat combustion in the puparium, to carbohydrate combustion upon emergence. During the 48 hours after emergence the adult R.Q. increases to more than 1.0 irrespective of whether the flies are starved or fed on sugar and water. This value could be due to experimental error but Chadwick (1947) obtained pre-flight R.Q. values in excess of 1.0 for Drosophila and attributed this to the conversion of carbohydrate to fat by flies recently removed from their food medium.

In the newly emerged adult the RDW represents about 24% of the initial live weight which is no different from the relationship in the pupa. The fat content, however, represents 27% of the RDW of the fly. This is a higher value than that obtained for the pupa since the puparial shell is not now included.

The puparial shell contains no measurable amount of fat and therefore lowers the estimate of fat as a percentage in the pupa.

Table II indicates that when adults are starved to death their fat contents fall to around 5% of the RDW. Survival is approximately 1 day longer in the presence of water, and fat contents fall below 5%. The fall in fat content from 27% to 5% represents a consumption of approximately 0.33 mg fat for a fly weighing 6.3 mg at emergence. We have found that the resting oxygen consumption of the unfed adult male C. capitata falls from about 2.5 $mm^3/mg/h$ to about 1.7 $mm^3/mg/h$ during the 48 hours following emergence. At 72 hours, more than 50% mortality has occurred. At this rate the 0.33 mg fat would be exhausted in 48 hours. Since the flies survive for approximately 24 hours longer than this, and their oxygen consumption increases greatly with activity, other reserves must be available to them. This view is supported by the high R.Q. values reported for the unfed adults.

Fat contents are maintained at between 12.8% and 16.8% of the RDW in adults which have fed (Table II). This means that the initial high fat content forms a utilizable reserve in the newly emerged adult. However, nothing is known of the carbohydrate reserves of the adult medfly.

Temperature, flight activity, and the extent of initial reserves will determine the survival potential of newly emerged medflies when released in the field. Water reserves must also be included here. However, there is no information in the literature on the water balance of medflies, and the significance of desiccation as a cause of mortality in the field is not known.

TABLE II. FAT CONTENTS OF ADULT MEDFLY SUBJECTED TO DIFFERENT TREATMENTS AT 25°C AND 75% r.h. AFTER EMERGENCE

Treatment	No. of flies (♂♂)	Fat content (%) non-fatty dry wt.
Killed immediately after emergence	100	27.5
Starved to death	100	6.2
	92	5.2
Starved to death in presence of water	100	5.0
	88	3.6
Fed 5 days on sugar and water	92	16.8
Fed 5 days on normal diet and water	92	12.8

Our own work has shown that reserves of newly emerged adult medflies can be significantly altered by different treatments during pupal development. Of particular note is the fact that the fat reserves of flies emerging from pupae incubated at temperatures above 25°C can fall close to those of flies which have died of starvation. This fact immediately calls in question the value of the practice of synchronization of pupal development by incubating at high temperatures. Further studies will be necessary to determine the safe low-temperature limit. Meanwhile, further studies on the effect of light upon the mature 3rd instar larva, when it leaves its medium to pupate, as a means of synchronizing adult emergence are clearly indicated by the results of Myburgh (1963).

The fact that the adult medfly will survive on sugar alone for some time after emergence, but dies more rapidly when fed on diets containing no sugar, does not justify the statement that adult medflies emerge with little or no stored energy resources (Keiser and Schneider, 1969). The tests were conducted within the very wide limits of 21-27°C and 50-89% r.h. and no information on treatment during the larval and pupal stage was given.

Until a more serious attempt is made to study in quantitative terms the physiological relationships of the medfly to its environment, much of the empirical work on medfly mass rearing and the sterile-male technique will remain difficult to interpret, and of limited value in predicting the success of an eradication campaign.

REFERENCES

BURSELL, E. (1959). The water balance of tsetse flies, Trans. R. ent. Soc. Lond. 111, pp. 205-35.

BURSELL, E. (1964). "Environmental aspects: humidity", in Physiology of Insecta (M. ROCKSTEIN, Ed.), Academic Press, New York, vol. 1, pp. 323-36.

CHADWICK, L.E. (1947). The respiratory quotient of Drosophila in flight, Biol. Bull. mar. biol. Lab., Woods Hole 93, pp. 229-39.

GILMOUR, D. (1961). The Biochemistry of Insects, Academic Press, London & New York.

KEISER, I., SCHNEIDER, E. (1969). Need for immediate sugar and ability to withstand thirst by newly emerged oriental fruit flies, melon flies, and Mediterranean fruit flies untreated or sexually sterilized with gamma radiation, J. econ. Ent. 62, pp. 539-40.

KEISTER, M., BUCK, J. (1964). "Respiration: some exogenous and endogenous effects on rate of respiration", in Physiology of Insecta (M. ROCKSTEIN, Ed.), Academic Press, New York, vol. 3, pp. 617-58.

MITCHELL, S., TANAKA, N., STEINER, L.F. (1965). Methods for mass culturing oriental, melon, and Mediterranean fruit flies, Rep. agric. Res. Serv., U.S. Dept. Agr., ARS 33-104, pp. 1-22.

MOURIKIS, P.A. (1965). Data concerning the development of the immature stages of the Mediterranean fruit fly (Ceratitis capitata (Wiedemann)(Diptera: Trypetidae) on different host-fruits and on artificial media under laboratory conditions, Annls Inst. phytopath. Benaki 7, pp. 59-105.

MYBURGH, A.C. (1963). Diurnal rhythms in emergence of mature larvae from fruit and eclosion of adult Pterandrus rosa (Ksh.) and Ceratitis capitata (Wied.), S. Afr. J. agric. Sci. 6, pp. 41-46.

SACANTANIS, K.B. (1954). Méthode d'élevage en laboratoire de la mouche des olives (Dacus oleae Gmel.), Revue Path. vég. Ent. agric. Fr. 32, pp. 247-57.

WIGGLESWORTH, V.B. (1949). The utilization of reserve substances in Drosophila during flight, J. exp. Biol. 26, pp. 150-63.

WIGGLESWORTH, V.B. (1965). The Principles of Insect Physiology, 6th Edn, Methuen, London.

RECENT RESEARCH IN HAWAII
ON THE MEDITERRANEAN FRUIT FLY

D. L. CHAMBERS, K. OHINATA, R. T. CUNNINGHAM
Entomology Research Division,
Agricultural Research Service,
United States Department of Agriculture,
Honolulu, Hawaii, United States of America

Abstract

RECENT RESEARCH IN HAWAII ON THE MEDITERRANEAN FRUIT FLY.

The feasibility of the sterile-insect technique as a method of suppression or eradication has been demonstrated for several fruit fly species. However, the competitiveness of the sterile insect is apparently less than that of its native counterparts. Current research at the Hawaiian Fruit Flies Investigations Laboratory is directed towards understanding the effects of the sterile-insect technique on this insect in order to improve the methods of application. Different types of methods of evaluation, improvement in feeding larvae and adults, and effects on the fly of radiation, packing, marking and release, are discussed.

1. INTRODUCTION

The use of sterile insects to eradicate populations of pests was conceived by Knipling (1955) and applied successfully for the first time in 1954 in campaigns against the screw-worm fly, Cochliomyia hominivorax (Coquerel), on the island of Curaçao (Baumhover et al. (1955)) and then in 1958 and 1959 in the south-eastern United States (Knipling (1960)). Subsequently, the potential of the method against other insect species, particularly fruit flies, Tephritidae, was recognized. As a consequence, personnel of the Hawaiian Fruit Fly Investigations Laboratory in Honolulu, Hawaii, used the method to eradicate the melon fly, Dacus cucurbitae Coquillett, from Rota in 1962-1963 (Steiner et al. (1965)) and the oriental fruit fly, D. dorsalis (Hendel), from Guam in 1963 (Steiner (1969)) [after an unsuccessful attempt had been made to eradicate it from Rota (Steiner et al. (1962))]. Also, a campaign is presently in progress to eradicate the melon fly from Guam (Chambers et al. (1970)).

The feasibility of eradication by the method of sterile-insect release has thus been proven against restricted, isolated populations of the melon fly and of the oriental fruit fly. However, the method has not been fully demonstrated against the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), though the first of the field tests of the method with Tephritidae were directed against this species, which is a pest of particular concern around the world because of its wide range, its potential for destruction, and its limiting effect on the production and movement of agricultural produce. In this attempt (1959 and 1960), Steiner et al. (1962) placed more than 18 million irradiated pupae in release stations spaced throughout an area of 31 km^2 on the island of Hawaii that contained wild Jerusalem cherry and an abandoned deciduous fruit orchard. The lack of isolation, and logistic and other difficulties, prevented any decisive eradication of the insect; however, the numbers of larvae found infesting

fruit samples were reduced. Subsequently, researchers came to the conclusion that the Mediterranean fruit fly is probably less amenable to sterilization by irradiation than the larger Dacus species (though there is little published information) and less able to compete with its native counterparts after laboratory rearing, irradiation of pupae, packaging, shipping, and release than are the other species. Research at the Honolulu and Hilo laboratories is now underway to obtain information about the effects of the method on the insect, so that techniques can be improved. The studies in progress include investigations of new methods of evaluating competitiveness and vigour, improvement of genetic stock, improvement in methods of mass rearing the flies, determination of the effects of the dose of irradiation and of the age of the flies at the time of irradiation on performance, the nutritive requirements of young adults, the effects of methods of packaging and distribution on adult flies, and methods of marking adult flies as well as the effects of such marking. Since the research is still in progress, the present paper is not a final report of results.

2. EVALUATING COMPETITIVENESS AND VIGOUR

Estimates of the competitiveness and vigour of flies prepared for release are made by comparing the mating or pairing frequency of treated versus untreated caged flies, by measuring vigour in release-recapture tests, or by measuring vigour directly on a flight mill.

The preliminary evaluations are made directly by placing known populations (various ratios) of treated males and untreated flies of both sexes in small 28 dm^3 cages. Eggs are collected periodically, and the hatch is recorded and used to measure the percentage suppression of fertility.

A similar test can be made in flight cages, but we prefer to use these larger cages to make another type of test that provides an additional estimate of the effect of treatment on mobility, vigour, aggressiveness, and selective mating (Holbrook and Fujimoto (1970)). Therefore, known numbers, usually 100 each, of treated and untreated males, and treated and untreated females are introduced into an 18 m^3 cage that has been placed in a sheltered outdoor location. In alternate replications, the treated or untreated flies are marked with a fluorescent dye. When mating commences pairs in copula are captured in vials and the participation of treated versus normal males can be recorded by noting the presence or absence of dye.

Release-recapture tests in the field, using marked flies, are invaluable tools for estimates of population, fly movement, longevity, and overflooding ratios. However, such tests cannot give a reliable estimate of vigour since the data are invariably compromised by test conditions. The flight mill designed by Chambers and O'Connell (1969) to measure flight capability in the laboratory and used in preliminary testing in Mexico City was tried in Hawaii and found applicable for the three species there. The permanent test equipment now being constructed will consist of 12 mills which will allow simultaneous measurement of 6 normal and 6 treated flies. Each mill has a vertical pinion that rotates freely in a magnetic field so there is minimum friction. The flies are harnessed to horizontal rotor arms that describe a circle of 1 m diam. Each rotation causes a small reflector to transmit light (by reflection) to a photocell receptor which actuates a

counter. Simultaneously, a specially designed circuit transmits an impulse to a magnetic tape recorder that can record the data from all 12 mills at once. Thus, the digital counters will show the total distance travelled. Meanwhile the more subtle differences, such as changes in flight rate and frequency and duration of resting periods, will be processed by computer. A program will instruct the computer to count, plot, and analyse the data stored on the magnetic tape. We hope to use the system to obtain much valuable information about the effect on vigour of such varied factors as irradiation and chemical sterilization, age and reproductive status, treatment with hormones, sublethal doses of toxicants, nutritional status and history, and cultural and endemic strains.

3. IMPROVEMENT OF GENETIC STOCK

An insect culture started from a native population may need 10 - 20 generations before it is sufficiently adjusted for easy laboratory cultivation but this adaptation may result in a strain very different from its progenitors. Therefore, in both Honolulu and Mexico City, an attempt has been made to maintain a semblance of a native gene pool by irregular introduction into the laboratory culture of native flies collected from hosts in the field. The irregular introduction, the small numbers, and the probable lack of participation of the native flies in mating and oviposition, mean that little if any significant impact is felt by the gene pool. A more effective approach would be selection of the laboratory culture for 'desirable' traits. Our initial attempt at such selection consisted of periodically isolating those adults (confined in 28 dm^3 cages) which, when startled, immediately flew through an opened shutter into another cage (placed above the first cage). Such a trait would be desirable in our laboratory strain of Mediterranean fruit flies because the culture tends to sedentary behaviour. However, the startle trait was not reproducible so we suspended this selection after a few generations. Instead, in our new effort, we have changed our rearing procedures so that the flies must fly to participate in feeding, mating, and oviposition, that is, such activities can take place only in the centre of a large (about 3 m^3) cage. After about 20 generations, we expect to compare the selected line with the normal laboratory stocks. If genetic improvement proves possible by this method, it should be feasible to arrange for routine selection of the rearing stock.

4. NUTRITION AND IMPROVEMENT OF MASS CULTURE METHODS

Nadel (1970) reviewed current methods of mass rearing Ceratitis species. The most significant improvement in our method has been the increase in the content of water in the diet made possible by the use of a water binding agent. This greater amount of water reduces heating of the medium by evaporative cooling, so larval recovery is improved and the amount of time to recover 90% of the larvae through popping is reduced from 4 to 3 days. Also, elimination of the practice of manual sifting of mature larvae from spent medium saves considerable time.

However, before attempting additional improvements in mass-rearing techniques, we are working to establish norms for larval and pupal size,

weight, rate of development, and percentage survival at standard conditions. Once such parameters are established, we can evaluate flies reared under a variety of test conditions.

In addition, we need a completely soluble defined or semidefined diet that could be applied to a re-usable, inert substrate capable of supporting the feeding larvae. Such an improvement has been partially realized by saturating any of several inert fibrous supports such as terry cloth, plastic floor coverings, or artificial grass with a yeast suspension.

5. EFFECTS OF IRRADIATION DOSE AND OF FLY AGE AT TIME OF IRRADIATION ON PERFORMANCE

Katiyar (in IAEA (1965)) and Katiyar and Valerio (1964) reported that sterilization by irradiation reduced the mating competitiveness of male Mediterranean fruit flies. Also, Holbrook and Fujimoto (1970) found that 8- to 10-day-old males irradiated with 10 krad as pupae 2 days before adult eclosion were about 50% as competitive as normal males though the females were unaffected. In our test of mating competitiveness in large outdoor cages, we found that males treated 2 days before eclosion with doses of 2.5 (ca. 93% sterile) or 5 krad (ca. 97.5% sterile) mated about 90% as frequently as normal males and that those treated with 7.5 (ca. 99% sterile) or 10 krad (99.9% sterile) mated about 65% as frequently as normal males; irradiated females, no matter what the dose, mated about 85-90% as frequently as normal females. Also, we studied flies treated with 3 or 7 krad to determine whether sterility caused by the substerilizing doses could be transmitted to the progeny. As expected, the egg hatch for the various male and female outcrosses and sibling crosses of F_1 and F_2 progeny originating from male outcrosses did not provide any evidence that sterility could be transmitted to progeny of flies irradiated with these doses 2 days before eclosion. However, in 18 m^3 outdoor cage tests, males or females treated at 10 krad 1 or 2 days after emergence were fully competitive with normal males or females for at least the first 10 days of adult life. This superiority in the competitiveness of flies irradiated as adults over those irradiated as pupae was corroborated in small cage tests and also in tests designed to determine sperm transfer. When the ratio of treated males to normal males to normal females was 9 : 1 : 1 in 28 dm^3 cages, the mean egg hatches over 30 days in a replicated test were 90.9% for the control (normal females and normal males only), 54.5% for females mated with males irradiated 2 days before eclosion, and 24.6% with males treated 2 days after eclosion (Table I). Moreover, when the spermathecae of 33 − 48 females (each placed for 3 - 4 days with one normal male or one male from one of the treatment categories) were checked for abundance of sperm, at least a trace of sperm was found in 96%, 41% and 89% of the females paired with normal males, males irradiated 2 days pre-emergence, and males irradiated 2 days post-emergence, respectively (Table II).

Irradiation may also reduce the effectiveness of male medflies in matings subsequent to the first. At Hilo, constant observation of pairs in small containers showed that flies of the laboratory strain emerging from pupae sterilized 2 days before adult eclosion with 10 krad of gamma irradiation mated about half as frequently and half as long as fertile males. Moreover, the courting period of these sterile males averaged one hour

TABLE I. POPULATION SUPPRESSION IN SMALL CAGES BY
MEDITERRANEAN FRUIT FLY MALES TREATED WITH 10 krad OF
GAMMA IRRADIATION 2 DAYS BEFORE OR 2 DAYS AFTER ECLOSION

Cage population I♂♂ : N♂♂ : N♀♀	Irrad. as:	Mean % hatch from 8 eggings over 1 month period	Mean % fertility	Mean % reduction in fertility
0 : 1 : 1		91.2		
9 : 1 : 1	Pupae 2 days before eclosion	54.5	59.7	40.3
9 : 1 : 1	Adults 2 days after eclosion	24.6	26.9	73.1

Notes: I = irradiated, N = normal. Tests done in 1 ft³ cages; 4 replications/treatment;
25 N♂♂ and 25 N♀♀/treatment.

TABLE II. COMPARATIVE SPERM TRANSFER BY NORMAL
MEDITERRANEAN FRUIT FLY MALES AND BY MALES TREATED WITH
10 krad GAMMA IRRADIATION 2 DAYS BEFORE OR 2 DAYS AFTER
ECLOSION

Treatment of males	Total no. of females dissected [a]	% of total no. of females dissected having indicated rating of sperm abundance [b]			
		0	1	2	3
Normal	48	4.2	5.3	24.2	66.3
10 krad 2 days before eclosion	33	58.5	13.8	4.6	23.1
10 krad 2 days after eclosion	43	10.7	10.7	23.8	54.8

[a] Each male in test provided with 1 virgin female at 3 - 4 days' interval over a month.

[b] Subjective ratings of sperm in spermatheca as follows: 0 = none, 1 = trace, 2 = few, 3 = abundant.

longer than that of fertile males, the sterile males took longer to mature
sexually, and the potency of sterile males, unlike that of fertile males,
decreased significantly after the adults were about 2 weeks old, from
observations of mating frequency and duration. Tzanakakis et al. (1968)
found that with the female olive fly, Dacus oleae (Gmelin), re-mating
frequency of the female was apparently affected by a material(s) transferred
by the male during coitus. Therefore, at our Hilo laboratory, individual

medflies are being mated and then later re-exposed to the opposite sex.
When the females accept re-mating, subsamples are dissected to determine
the content of sperm. If the re-mating of female medflies is a function of
the amount of sperm or other material transferred or its rate of depletion,
reduced transfer of sperm by sterile males of reduced potency would
affect the mating frequency of the female, and more vigorous males would
be necessary for the release program.

6. NUTRITIVE REQUIREMENTS OF YOUNG ADULT FLIES

During the 1962 and 1963 eradication campaigns in the Pacific islands,
the standard nutrition supplied to the flies emerging in the containers was
a mixture of honey, sugar and water (1 : 1 : 1). Harris et al. (1968) found
that melon flies confined 3 weeks in distribution boxes survived better on
this mixture or on a mixture containing sugar and water than on a mixture
of honey and water. However, logistics and the need to deliver flies of
optimum quality to the release site dictate that flies should emerge from
the distribution boxes as soon as possible after adult emergence. Moreover,
even in the Pacific where the flies had to be shipped from Honolulu to Guam
and Rota, the adult flies were usually not in the boxes more than 48 hours.
The recent report by Keiser and Schneider (1969) is therefore pertinent to
a release program. They found that newly emerged adults of all three fruit
fly species require nothing but sugar for the first 72 hours of life; melon
flies and Mediterranean fruit flies suffered less than 2% mortality in 72 hours
and less than 10% mortality in 96 hours when nothing but sugar was avail-
able. The oriental fruit fly was somewhat less tolerant of the deprivation
of water. However, sugar, not water, is the prime requirement for early
survival of all three species.

We have not yet tested the vigour and competitiveness of flies held on
the various diets. However, the effect of nutrition on the effectiveness of
sterile flies will be evaluated; perhaps flies deprived but not physiologically
damaged may enter the environment more vigorously than replete adults.

7. EFFECTS OF PACKAGING ON ADULT FLIES

Harris et al. (1968) studied the emergence of about 4500 young adult
fruit flies confined in a drop box measuring $8.9 \times 15.2 \times 26.2$ cm and
divided into 77 interior cells: the survival of melon flies and oriental fruit
flies was reduced 35 and 44%, respectively, by 3 weeks' confinement, and
mutilation was increased about 5-fold, to 8 - 10%. Nadel et al. (1967)
devised a method of releasing adults irradiated as pupae in paper bags
(containing wood wool) which could be easily distributed from aircraft.
Also, Holbrook et al. (1970a) loaded No. 12 Kraft® paper bags partitioned
with X-shaped cardboard inserts with 3000 pupae/bag and reported that
about 50% of the Mediterranean fruit flies escaped immediately when the
containers were opened and that about 90% of the melon flies escaped; they
also reported mutilation of both species to be less than 5%. However,
when these same bags were recently used by the Guam Department of
Agriculture in its eradication program against melon flies, they found
mutilation in a few randomly selected bags to be as high as 33%, and this
value did not include the damage to the flies that occurs when the opened

bag enters the slipstream of the aircraft. Clearly, losses due to damage and mutilation by packaging must be minimized, particularly for Mediterranean fruit flies whose vigour and competitiveness are in question. We are therefore re-evaluating the possibility of distributing loose pupae from aircraft, a method discounted because of the high mortality that occurs from predation and solar heat (Nadel et al. (1967)).

In our tests, about 87% of the Mediterranean fruit fly pupae easily survived a 60-m free fall drop from a balloon to grassy fields; 62% and 41% of those dropped on forest and bare rocky ground, respectively, emerged. Since the free-falling pupae reach a terminal (Stoke's law) velocity of about 6 m/s after they fell about 3 m, they could be dropped from any altitude. Subsequently, 300 000 pupae and a similar number of adults packaged in bags as described above were dropped from a light aircraft flying at an altitude of 90 m over varied terrain; in 3 weeks, 42% as many flies were recovered from the pupal, as from the adult distribution. Therefore, the relative advantages of the two systems will depend on the type of terrain, the savings in packaging costs, and the quality of the emerging flies.

8. METHODS OF MARKING ADULT FLIES AND EFFECTS

Roan (1952) described methods of marking fruit flies with radioactive phosphorus for studies of movement in the field. In 1962 and 1963, Steiner (1965) used topically applied dyes or dye-coated pupae substrates (an adaptation of a method used in 1958 to mark houseflies, Musca domestica L., at the Insects Affecting Man and Animals Research Laboratory then at Orlando, Florida) and described a method of identifying the marked flies. Shaw et al. (1966) suggested coloured lacquer for marking. Holbrook et al. (1970b) evaluated the mating competitiveness of dye-marked flies by using the mating pair-capture technique and found that the mating competitiveness of otherwise untreated Mediterranean fruit flies marked with orange, red, yellow, or green Day-Glo® fluorescent powders was not impaired.

TABLE III. MATING ABILITY OF DYED AND UNDYED MEDITERRANEAN FRUIT FLY MALES IRRADIATED WITH 10 krad 2 DAYS BEFORE OR 2 DAYS AFTER ECLOSION

| Age of flies (days) | % of dyed and undyed irradiated males mating with normal females | | | |
| | Males irradiated 2 days before eclosion | | Males irradiated 2 days after eclosion | |
	Dyed	Undyed	Dyed	Undyed
5	49.2	50.8	52.2	47.8
10	42.2	57.8 [a]	42.9	57.1 [a]
15	42.8	57.2 [a]	46.6	53.4

[a] Difference between means significant at 5% level, according to Student's t-test. Each value is a mean of 4 replicates.

However, the combination of irradiation and dye marking appeared to be detrimental to the mating competitiveness of male flies during recent evaluations of the effect of radiation dose. We therefore made direct comparisons of the mating abilities of dyed (Day-Glo red) and undyed irradiated males (treated with 10 krad either 2 days before or 2 days after adult eclosion) by placing 100 of each with 100 normal females in outdoor cages and capturing and identifying the pairs in copula. Table III shows that at 5 days of age there was no significant difference in the pairings of dyed and undyed males irradiated at either stage. However, at 10 and 15 days of age, the competitiveness of males irradiated as pupae was impaired by the presence of dye: irradiation and dyeing of adult males reduced competitiveness at 10 days of age but not at 15. Though the dye appeared to be only slightly detrimental, we have shown that such effects can be cumulative and must be considered in a release program.

9. EFFECT OF DOSE OF IRRADIATION ON EGG LAYING

Nadel (IAEA, Vienna, personal communication) reported that eggs can be produced by female medflies irradiated as pupae 24-48 hours before emergence with doses of as much as 12 krad. However, all our data indicate that egg laying by laboratory-reared females treated 2 days before emergence can be prevented with a dose of 2.5 - 3 krad. In a recent test made to resolve these differences, we treated about 750 females each with 3.8, 5.7, 7.8, 9.8, or 12.1 krad 48 ± 6 hours before emergence and confined them with an equal number of males irradiated at the same dose: they laid no eggs in 7 eggings over 24 days; however, flies treated at 1.8 krad laid about 5000 eggs (3.4% hatch) compared with 185 000 (90.3% hatch) laid by untreated females crossed with untreated males. [These pupae were treated in a $6.3 \times 6.3 \times 1.3$ cm container in a section of the irradiation chamber (pool-type unit, dose rate approximately 4500 rad/min) where the maximum-to-minimum dose range was less than 10%, as determined by Fricke dosemeters.]

In another study, 10-day-old females treated with 10 krad as pupae just before adult emergence, as 1-day-old adults, or as 2-day-old adults laid about 0.4%, 7% and 45% as many eggs/fly as normal females of the same age.

10. CONCLUSIONS

We have described a few of our current research programs with the Mediterranean fruit fly. Those selected for description indicate the direction of current research in Hawaii. The successes achieved in the South Pacific islands demonstrate amply that the method of sterile-insect release is feasible for eradication of fruit flies and that the failures there and in Hawaii are the result, at least in part, of logistic and procedural difficulties. However, as we have attempted to overcome these difficulties, we have encountered new problems. Thus, we are demonstrating the need for a research program of both breadth and depth. Development of the techniques of culturing, treatment and release have

first priority, but the mechanics of a release program must be supported by knowledge about the interaction between the insect and its treatment and the effect on performance in the release program.

Note

Mention of a proprietary product does not constitute endorsement of the product by the U.S. Department of Agriculture.

<div align="center">REFERENCES</div>

BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, D.E., NEW, W.D., DUDLEY, F.H., BUSHLAND, R.C. (1955). Screw-worm control through release of sterilized flies, J. econ. Ent. 48 (4), pp. 462-6.

CHAMBERS, D.L., O'CONNELL, T.B. (1969). A flight mill for studies with the Mexican fruit fly, Ann. ent. Soc. Am. 62 (4), pp. 917-20.

CHAMBERS, D.L., SPENCER, N., TANAKA, N. (1970). "Sterile-insect technique for eradication or control of the melon fly and oriental fruit fly: review of current status", these Proceedings.

HARRIS, E.J., MITCHELL, W.C., BAUMHOVER, A.H., STEINER, L.F. (1968). Mutilation and survival of sterile oriental fruit flies and melon flies emerging in drop boxes, J. econ. Ent. 61 (2), pp. 493-6.

HOLBROOK, F.R., FUJIMOTO, M.S. (1970). Mating competitiveness of unirradiated and irradiated Mediterranean fruit flies, J. econ. Ent. 63, pp. 1175-76.

HOLBROOK, F.R., STEINER, L.F., FUJIMOTO, M.S. (1970a). Holding containers for melon flies and Mediterranean fruit flies for use in sterile fly aerial releases, J. econ. Ent. 63, pp. 908-10.

HOLBROOK, F.R., STEINER, L.F., FUJIMOTO, M.S. (1970b). Mating competitiveness of Mediterranean fruit flies marked with fluorescent powders, J. econ. Ent. 63, p. 454.

IAEA (1965). Advances in Insect Population Control by the Sterile-Male Technique (Report Panel Vienna, 1964), Tech. Rep. Ser. No. 44, IAEA, Vienna, section III B, pp. 20-21 (Laboratory studies in Costa Rica).

KATIYAR, K.P., VALERIO, J. (1964). Efecto causado por la introduccion de machos esterilizados por radiacion gamma, en una poblacion normal de moscas del Mediterraneo (Ceratitis capitata), Turrialba 14 (4), pp. 211-2.

KEISER, I., SCHNEIDER, E.L. (1969). Need for immediate sugar and ability to withstand thirst by newly emerged oriental fruit flies, melon flies, and Mediterranean fruit flies untreated or sexually sterilized with gamma radiation, J. econ. Ent. 62 (3), pp. 539-40.

KNIPLING, E.F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males, J. econ. Ent. 48 (4), pp. 459-62.

KNIPLING, E.F. (1960). The eradication of the screw-worm fly, Scient. Am. 203 (4), pp. 54-61.

NADEL, D.J. (1970). Current mass rearing techniques for the Mediterranean fruit fly, these Proceedings.

NADEL, D.J., MONRO, J., PELEG, B.A., FIGIDOR, H.C.F. (1967). A method of releasing sterile Mediterranean fruit fly adults from aircraft, J. econ. Ent. 60 (4), pp. 899-902.

ROAN, C.C. (1952). Tagging oriental fruit flies with radioactive phosphorus for field-movement studies, J. econ. Ent. 45, p. 826.

SHAW, J.G., PATTON, W.P., SANCHEZ-RIVIELLO, M., SPISHAKOFF, L.M., REED, B.C. (1966). Mexican fruit fly control, Calif. Citrogr. 51 (5), pp. 209-14.

STEINER, L.F. (1965). A rapid method for identifying dye-marked fruit flies, J. econ. Ent. 58 (2), pp. 374-5.

STEINER, L.F. (1969). Control and eradication of fruit flies in citrus, Proc. Int. Citrus Symp., Riverside, Calif., March 1967, vol. 2, pp. 881-7.

STEINER, L. F., MITCHELL, W. C., BAUMHOVER, A. H. (1962).   Progress of fruit fly control by irradiation sterilization in Hawaii and the Marianas Islands, Int. J. appl. Radiat. Isotopes 13, pp. 427-34.

STEINER, L. F., HARRIS, E. J., MITCHELL, W. C., FUJIMOTO, M. S., CHRISTENSON, L. D. (1965). Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58 (3), pp. 519-22.

TZANAKAKIS, M. E., TSITSIPIS, J. A., ECONOMOPOULOS, A. P. (1968). Frequency of mating in females of the olive fruit fly under laboratory conditions, J. econ. Ent. 61 (5). pp. 1309-12.

APPLICATION OF THE STERILE-MALE TECHNIQUE IN MEDITERRANEAN FRUIT FLY SUPPRESSION

A follow-up experiment in Nicaragua

R. H. RHODE[*]
Apartado Postal 3628,
San José, Costa Rica

Abstract

APPLICATION OF THE STERILE MALE TECHNIQUE IN MEDITERRANEAN FRUIT FLY SUPPRESSION:
A FOLLOW-UP EXPERIMENT IN NICARAGUA.

A follow-up experiment designed to demonstrate Mediterranean fruit fly suppression in a 48-km² area in the region of Carazo, Nicaragua, is discussed. This release area was surrounded by a 2-km wide band which received several chemical treatments by applying a 9:1 mixture of protein hydrolysate and technical malathion. Also, two check areas were included. Sterile flies were released by plane about 4 days a week, approximately 11 million flies per flight. Results indicate a clear suppression of the Mediterranean fruit fly when comparing the number of wild flies, fruit infestation and percentage egg hatch in the release area with the check area.

Large-scale field application of the sterile-male technique to suppress or eradicate polygamous fruit-fly species closely followed the successful elimination of the screw worm, Cochliomyia hominivorax (Coquerel), from the island of Curaçao by this method (Baumhover et al. (1955)). In 1960 Steiner et al. (1962) obtained a 90% reduction in Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), infestations over an area of approximately 32 km² in Hawaii. Eradication of the melon fly Dacus cucurbitae Coquillett from the 78-km² island of Rota was achieved in 1962 and 1963 (Steiner et al. (1965)).

In 1965 the International Atomic Energy Agency through the United Nations Development Programme (Special Fund) initiated a cooperative program with Organismo Internacional Regional de Sanidad Agropecuaria to determine the feasibility of eradicating the medfly from Central America using the sterile-male technique. Other contributing organizations were the Ministries of Agriculture of Nicaragua, Costa Rica and Panama and the U.S. Department of Agriculture. Supporting research was carried out by the U.S. Atomic Energy Commission group at the International Institute of Agricultural Sciences at Turrialba, Costa Rica. Aircraft and other facilities of the Nicaraguan and U.S. Air Forces were made available to the project.

In 1967 an attempt was made to eradicate the medfly from 120 km² in Nicaragua. However because of high wild fly populations and lack of a properly isolated test site, this effort was without success.

The present paper presents a follow-up experiment designed to demonstrate medfly suppression on a reduced scale within the same work area in Nicaragua.

---

[*] IAEA Expert.

The test was conducted in a 48-km^2 area situated in the Department of Carazo, Nicaragua. In this highland region approximately 600 km^2 are planted to coffee grown under natural forest cover and citrus, both of which are principal medfly hosts. Another host fruit elsewhere heavily attacked but of apparently minor importance in Carazo is rose apple, Eugenia jambos (L.), the trees of which are used as wind breaks. With the exception of medfly infestations in coffee planted on the slopes of 1300-m-high Mt. Mombacho lying 22 km east of the test site, the Carazo region is about 100 km from the nearest host environment in which extensive infestations could develop. The experimental area is situated near the southern limits of the coffee region on comparatively level ground which slopes gradually upward towards the north. Here the terrain changes abruptly into a series of deep narrow canyons. West of the sterile-fly release site the land falls off to the Pacific Ocean and on the east towards Lake Nicaragua 30 km distant. Winds predominantly from the east and north-east are constant most of the year but increase in intensity during the dry season from November to May. Sweeping across Lake Nicaragua they create considerable air turbulence over Carazo and constitute a hazard to low-level flying. Average annual precipitation is around 150 cm.

The main coffee harvest in Carazo extends from September to January but some early maturing coffee is picked in August. Infestations in scattered coffee berries have been found in June. Mandarin oranges, preferred by medfly over other citrus species present, are in evidence from December to June and most abundant in March.

Medfly host fruit density is high within the test area and most of its immediate surroundings. With these conditions it was impractical and beyond the financial resources of the program to attempt to create a completely isolated test situation by means of quarantines and aerial spray operations. However, it was believed that in accord with the experimental objective the sterile release site would receive adequate protection from migrating wild flies by periodic insecticide applications to a 2-km-wide barrier around the perimeter.

The bait spray formulation applied by air at the rate of about 1.4 litres per hectare consisted of a 9:1 mixture of protein hydrolysate and technical malathion.

Scheduled at 2-week intervals, the chemical treatments were intended to suppress wild fly build-up and to inhibit fly movement into the adjacent sterile-fly release zone. For economic reasons and considering that eradication was not an objective, the insecticide mixture was applied to one-third of the 72-km^2 border area by spraying swaths 30 m wide at 90-m intervals.

The material was applied by a single-engined plane flying 80 - 100 miles/h (128 - 160 km/h) at around 50 m altitude. Spray tank pressure was about 25 - 30 lb/in^2. Each spray boom had five nozzles with 12/64-inch (4.7625-mm) orifices. The spray droplets ranged in size from 0.5 to 4 mm and appeared well distributed. A guide plane assisted in the spray application.

Two 36-km^2 check areas were included in the experimental set-up. Check 1 was located about 12.5 km north-west and Check 2 was located 4 km east of the release site. Mandarin orange trees and coffee are present in all locations but mandarins are less abundant in Check 1. Mean elevations of the test site and Checks 1 and 2 are 600 m, 680 m and 430 m respectively. Unimproved roads transect these areas and except for

occasional brief periods most are transitable in the wet season with a 4-wheel drive vehicle. In the sterile-fly area 153 Steiner traps were distributed with 55 in Check 1 and 62 in Check 2. An additional 110 traps were maintained immediately outside these areas for detection purposes. All traps were assigned permanent locations. Trimedlure used as bait was applied to 2-cm-diam. cotton wicks and a small amount of chlordane-lindane powder was put in the bottom of each trap. Traps were examined at weekly intervals with the exception of those situated outside the experimental complex, which were serviced every two weeks.

At the inception of the experiment on 13 September 1968 a centre field irradiation dose of 9.8 kR (±13%) was administered to pupae in lots of 90 000. From October to late November the dose was lowered to 7 kR and 8 kR. It was assumed that less exposure would result in a more vigorous male which through increased sexual activity would compensate for the mean 3 or 4% residual male fertility found at these dosages.

In late November as a security measure the sterilization dose was raised to 9 kR for the duration of the experiment, as a result of evidence that some individual males may exhibit a higher degree of fertility. However, in laboratory tests differences in mating ability significant at the 5% level were observed between males treated with 6 kR, 8 kR and 10 kR of gamma radiations. At 9 kR average male fertility was found to be around 1.1%.

Female flies were released with the males because no practicable method suitable for large-scale operations is known to separate the sexes in the pupal stage. Pupae were in the irradiation canister less than 2 min and no anoxia effects were noted.

The age differential of developing pupae was reduced by collecting the mature larvae from the recovery cabinets every 8 hours. To insure a biological age of 7 to $7\frac{1}{2}$ days at the time of sterilization, pupal maturation was controlled by varying time and temperature relationships. At 20°C pupal development is about half that at 28°C.

After irradiation, fluorescent powders applied at the rate of 4g/l of pupae were used to mark the emerging flies for subsequent identification. Random samples of flies taken from the bags showed that on the average 99.6% were well marked on the body and/or ptilinum. When viewed under ultra-violet light the sterile and unmarked wild flies were generally readily distinguished. In doubtful cases the flies' heads were dissected under a microscope to detect any enclosed fluorescent particles. Mating performance of the sterile males did not appear to be significantly affected by the powders.

From 4 to 6 thousand pupae were measured into a 5 kg Kraft paper bag. About 100 g of 6-mm-wide wood excelsior strips placed in the bag maintained rigidity and provided resting surfaces for the flies. This material was made from the wood of two local tree species: Alnus acuminata HBK and Daphnopsis seibertii Standl. A 30-cm length of 1.25-cm-diam. cotton wicking saturated with a mixture of honey, sugar and water in equal proportions provided food and moisture. The bags were sewn shut with a bag-closing machine and packed 24 to a cardboard box measuring about 57 cm × 57 cm × 42 cm. The boxes were stored in a single layer on latticed shelves in a semi-darkened and ventilated room maintained between 23°C and 25°C. After packing, the boxes were left open throughout the entire transport and release operations to facilitate dissipation of metabolic heat generated by the emerging flies.

Fly emergence began on the day of treatment and was nearly completed on the morning of the scheduled release 2 days later. Adult eclosion averaged 90.1% throughout the test period.

About 4 times per week C-47 aircraft of the Nicaraguan Air Force arrived in San José, Costa Rica, to pick up the sterile flies. The flies were transported to the airport in a well-ventilated truck and loaded without delay on the plane immediately before take-off. The plane's main cargo section could accomodate 90 boxes with sufficient excess space for a passageway along one side. At an average bag loading rate of 5000 pupae, approximately 11 million flies were released on each flight. To prevent the flies from overheating during the half-hour trip to the airport and later aboard the plane the stacked boxes were separated by 2-m lengths of 2.5 cm $\times$ 5 cm strips of lumber. As the jump door of the C-47 was always removed to accommodate the release chute, air circulation held internal box temperatures below 28°C.

The release plane traversed the experimental area in a north and south direction. With prevailing easterly winds, the cross-wind distribution obviated adjustments on each leg to maintain a uniform bag drop rate. Fly drops were usually made from 100 m to 160 m altitude at around 120 knot indicated air speeds. Under unusually turbulent conditions altitude was increased to 200 m or 230 m. Releases at altitudes lower than 100 m were impractical because the wide crowned forest trees interfered with reference point sightings. At altitudes above 160 m bag drift was excessive under windy conditions. On occasions bags were observed to fall an estimated 460 m from the flight path when released at around 230 m.

The pilot and flight supervisor employed a set of four identical topographical maps. The 1-km-distant flight lines plotted on each map were offset 250 m to the right of the lines on the preceding map. After flying all lines in a 1-3-2-4 map sequence sterile flies were distributed over the area along paths 250 m apart. To compensate for wind drift and to ensure sterile-fly coverage along the windward edge, flight lines were extended 750 m beyond the eastern boundary of the experimental site. A bag with flies was slit open on two sides as it left the release chute and dropped at approximately 100-m intervals along each line. With this distribution pattern a weekly average of 96.2% of the traps in the test area captured sterile flies. The 250-m distance between flight lines was arrived at as a result of previous experience in methods development. Aerial releases made at 500-m intervals in the test area gave evidence of irregular sterile-fly distribution. Also trap catches revealed that substantial numbers of sterile flies apparently tended to remain in the vicinity of where they left the fallen bags. Later in the program a ground dispersal test conducted in the experimental area demonstrated that over a 3-week period after release 83.8% of the sterile flies recaptured were taken within 250 m of a 6.2-km-long release line. Some individuals demonstrated greater flight capability. Two flies were recovered at a point 10 - 10.5 km and one fly at a point 11 - 11.5 km from the release line.

The effect of sterile males on the wild female population was measured by host fruit sampling. From October 1968 to February 1969 random samples of mature coffee cherries were taken each week except during January from a number of locations within the test site and Check 1. Fruits exhibiting oviposition puncture marks were dissected under a microscope and a count made of any larvae and/or non-eclosed eggs found. The eggs

were removed from the fruit and held 4 days on moist filter paper in Petri dishes to determine viability rates.

From 18 to 28 April random samples of ripe tree-picked mandarin oranges were taken from three locations in the test area and six in La Concepción during the last of the commercial harvest. Fruit samples were collected from citrus groves in which no medfly control spraying was done. The directions and distances in kilometres of the groves in the test area from their closest point to the bait-sprayed border zone were as follows: El Maderal 1.7 west, San Fernando 0.9 west and Las Carolinas 1.6 south. All the groves in La Concepción lie north of the border zone. That of R. Castro is nearest at 0.9 km. The others range from 1.1 to 1.3 km distant. Trap density in the vicinity of the fruit collecting sites was 2.1/km^2. The fruit samples taken from the test area and La Concepción were held to determine infestation rates at one central point in each of the respective areas. The mandarins, separated in groups according to their collecting sites, were spread in two layers over 2.5 cm of sand in shallow pits protected from the sun. The sand was placed over cotton sheets to prevent the larvae from entering the soil. To protect the fruit from insect predators a 30-cm-wide band of chlordane dust was spread around the perimeter of the 100 m^2 holding sites. At 8 to 12 days after collection each fruit was examined for mature larvae and the sand sifted with a fine mesh wire screen to recover the pupae.

In 1967 wild flies in the test area were lowest in September and October. As the host fruit season progressed, flies increased to a peak in January 1968 followed by a second higher peak in March (Table I). In anticipation of a similar wild fly population pattern during the 1968-69 season a spray application was given to the border zone on 28 December 1968. However, wild fly numbers in Check 1 were considerably less than those in the same area the previous December. A late-maturing coffee crop evidently was delaying the expected wild fly build-up which occurred in January 1968. Consequently spray operations were suspended until an upward trend in the wild population became apparent in order to reserve spray materials for the crucial period of high wild fly numbers. Through a misunderstanding a partial spray coverage was given the border area on 9 January 1969. In mid-January native fly populations showed evidence of rising. From 4 February to 20 March four spray applications were made at 2-week intervals. The final spray applied 9 April was delayed one week because of Holy Week festivities in Nicaragua. The suppressive effect of the bait sprays on wild fly build-up in the border zone is apparent (Table I). In spite of the slow wild fly population build-up in the 1968 - 69 host fruit season wild fly numbers peaked at nearly the same time as in 1967 - 68. During the week of 10 - 16 March 1968 the test area recorded 3.492 wild flies/trap/day and Check 1, 1.065. In 1969 from 9 - 15 March respective catches in the two areas were 0.063 and 1.140. For unknown reasons appreciable numbers of wild flies were not produced in Check 2. No wild flies were captured in Check 2 from September 1968 to January 1969. The period of its peak wild fly trap catches in March 1969 coincided with the other areas but reached only 0.030 flies/trap/day. Spot checks revealed no infestations in coffee or mandarin. Therefore this Check was rejected for comparison of fruit infestation data.

Wild fly populations were generally declining in the test area in September 1968. In the week preceding the initial sterile-fly release, wild

TABLE I. AVERAGE STERILE AND WILD FLY/TRAP/DAY CATCHES
IN 1968 - 69 AND WILD FLY CATCHES IN 1967 - 68 IN EXPERIMENTAL
AREAS

Month	Area	1968-69 Sterile	1968-69 Wild	1967-68 Wild
September	Test	4.48	0.044	0.022
	Border	0.56	0.012	
	Check 1	0.0	0.014	
	Check 2	0.0	0.0	0.076
	La Concepción			
October	Test	22.44	0.017	0.060
	Border	4.72	0.018	
	Check 1	0.0	0.007	
	Check 2	0.007	0.0	0.0
	La Concepción			
November	Test	32.85	0.012	0.568
	Border	3.75	0.001	
	Check 1	0.0	0.0	
	Check 2	0.014	0.0	0.105
	La Concepción	0.018	0.0	
December	Test	59.89	0.014	0.235
	Border	9.32	0.006	
	Check 1	0.0	0.003	0.115
	Check 2	0.006	0.0	0.155
	La Concepción	0.005	0.003	
January	Test	31.65	0.010	0.987
	Border	3.82	0.018	
	Check 1	0.0	0.018	0.164
	Check 2	0.0	0.0	2.497
	La Concepción	0.178	0.0	
February	Test	34.28	0.028	0.531
	Border	3.59	0.002	
	Check 1	0.0	0.092	0.239
	Check 2	0.002	0.005	0.212
	La Concepción	0.183	0.009	
March	Test	46.19	0.040	1.588
	Border	4.73	0.005	
	Check 1	0.0	0.549	0.656
	Check 2	0.019	0.025	0.468
	La Concepción	0.165	0.039	
April	Test	27.97	0.038	0.501
	Border	2.62	0.007	
	Check 1	0.0	0.347	0.117
	Check 2	0.003	0.013	0.227
	La Concepción	0.406	0.142	

fly captures were 0.044 flies/trap/day. After two weeks of releases the sterile-to-wild-fly ratio reached 116:1. Except for the penultimate week of the experiment when the ratio dropped to 238:1 overflooding with sterile flies exceeded 433:1. With occasional wide fluctuations, weekly sterile-to-wild-fly catches averaged 1338:1. Apart from variations in the sterile-to-wild-fly ratios caused by differences in numbers of wild flies caught, a few traps at times registered unusually high numbers of sterile flies. To account for some of these anomalies there was evidence that fallen bags were recovered and placed in coffee farms near where the traps were located. Wild flies appeared to be more prevalent in several rather well-defined islands within the test area. To prevent 'hot spots' of infestations from developing, additional sterile flies were dropped at irregular intervals over these areas. This was accomplished by releasing bags of flies rapidly while the plane described an increasingly tight spiral over the target site.

Approximately 8869 coffee cherries from the test area and 6775 from Check 1 were examined between October 1968 and February 1969. In fruits from the test area 267 eggs, of which 7 were fertile, and 20 larvae were encountered. The combined numbers of larvae and fertile eggs gave a 9.41% rate of viable medfly forms. The coffee cherries of Check 1 yielded 224 larvae and 41 eggs, of which 38 were fertile, for a viable form incidence of 98.87%. These data indicated that fertile wild medfly eggs and larvae were 90.48% less abundant in the test area than in Check 1. About 85% of the coffee infestation data was obtained during February from only 22.2% of the total amount of fruit examined in the entire period. At this time more eggs and larvae were found in relation to numbers of fruit collected probably as a result of the higher fly-to-fruit ratio expected late in the harvest season.

The data of February alone show that viable wild medfly forms were 97.11% less in the test area than in Check 1. From 2027 coffee cherries collected in the test area 5 larvae and 205 eggs, only one of which was fertile, were taken, representing a viable form rate of 2.86%. Fruits examined in Check 1 totalled 1309. Encountered were 222 larvae and 38 eggs, 35 of which were fertile, for a 98.85% rate of live forms.

Medfly infestations in mandarin oranges from Check 1 and the test area were not compared because the fruit harvest in Check 1 ended shortly after the mid-March wild fly population peak. In both La Concepción and the test areas the owners of commercial-sized mandarin orchards delayed the harvest until market prices improved. In March the fruit infestation potential represented by numbers of wild flies caught was almost identical in each area (Table I). At this time, with a generation occurring about every 6 weeks, eggs deposited in fruit during March would be recovered as pupae in April.

A total of 1134 kg of fruit was sampled in the test area and 1279 kg from the alternate check in La Concepción. The infestation data in Table II show that pupal recovery rates per pound of fruit averaged 90.5% less in the test area than in the check.

Approximately 1 119 410 210 sterile flies were released up to 3 May 1969 when the experiment ended. Sterile-male fly recovery from the test and border areas averaged 0.0927%/trap/km². Many of the sterile flies released or which drifted into the border zone undoubtedly were killed by the insecticide treatments. Movement of sterile flies from the test area into La Concepción and Check 2 was apparent (Table I). It can be assumed

TABLE II. MEDFLY INFESTATIONS IN MANDARIN ORANGE SAMPLES FROM THE TEST AREA AND FROM LA CONCEPCIÓN

Area	Location	Fruit (kg)	Pupae recovered	Pupae/kg fruit
Test	El Maderal	461	33	0.0716
	San Fernando	396	1	0.0025
	Las Carolinas	279	21	0.0753
			Average pupae/kg fruit:	0.0498
La Concepción	C. Martínez	30	46	1.5333
	A. Ortiz	102	1	0.0098
	J. Santos	147	4	0.0272
	S. Hernández	73	40	0.5479
	R. Castro	482	526	1.0913
	U. Carrasquilla	445	37	0.0831
			Average pupae/kg fruit:	0.5489

also that some wild flies migrated successfully into the test site. Another possible manner of wild fly introduction into the test area occurred in the mandarin season. During this period the three towns situated within the boundaries of the test area received considerable quantities of fruit from the major citrus producing region of La Concepción.

In spite of these adverse conditions the wild fly population in the test area increased only ×3.3 from its relatively low level in November to its peak in March. In contrast the wild fly populations of Check 1 and La Concepción increased ×183 and ×47.3 during the period from their first detectable levels in December to their respective peaks in March and April (Table I).

REFERENCES

BAUMHOVER, A. H., GRAHAM, A. J., BITTER, B. A., HOPKINS, D. E., NEW, W. D., DUDLEY, F. H., BUSHLAND, R. C. (1955). Screw-worm control through release of sterilized flies, J. econ. Ent. 48 (4), pp. 462-66.

STEINER, L. F., MITCHELL, W. C., BAUMHOVER, A. H. (1962). Progress of fruit fly control by irradiation sterilization in Hawaii and the Marianas Islands, Int. J. appl. Radiat. Isotopes 13, pp. 427-34.

STEINER, L. F., HARRIS, E. J., MITCHELL, W. C., FUJIMOTO, M. S., CHRISTENSON, L. D. (1965). Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58 (3), pp. 519-22.

THE PRE-RELEASE PHASE OF THE 1969 MEDITERRANEAN FRUIT FLY SUPPRESSION EXPERIMENT IN ITALY

G.H.S. HOOPER, D.J. NADEL
International Atomic Energy Agency,
Vienna

Abstract

THE PRE-RELEASE PHASE OF THE 1969 MEDITERRANEAN FRUIT FLY SUPPRESSION EXPERIMENT IN ITALY.

The various steps involved in the production, sterilization and shipment of sterile Ceratitis capitata (Wiedemann) for the 1969 suppression experiment on the island of Procida, Italy, are described. The results of tests, carried out on each shipment of pupae, to evaluate adult emergence and sterility are reported.

1. INTRODUCTION

In 1967 and 1968 the International Atomic Energy Agency (IAEA) co-operated with the Italian Ministry of Agriculture and the Comitato Nazionale per l'Energia Nucleare (CNEN) in experiments on the islands of Capri and Procida, Italy, to demonstrate suppression of wild populations of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), with the sterile-insect method (IAEA (1968), Nadel and Guerrieri (1969)). In 1969 a further co-operative experiment was conducted on Procida to confirm the applicability of the sterile-insect release method for control of C. capitata. The present paper is concerned with the pre-release phase of this experiment which was carried out by the IAEA Seibersdorf Laboratory, Seibersdorf, near Vienna, and was divided into two distinct components; firstly, the production, irradiation and shipment of all pupae for the experiment; and secondly, tests on each consignment of pupae to evaluate adult emergence and sterility of male and female flies. The various steps involved in both components of the pre-release phase are described.

2. REARING, IRRADIATION AND HANDLING OF PUPAE

Pupal production

The larval medium and techniques used in the rearing of the pupae have been described (IAEA (1965, 1969), Nadel and Peleg (1968)). However, the transfer of production of pupae to a new laboratory provided the opportunity for certain changes and improvements.

Pupae were collected daily in trays containing bran placed beneath the larval units. Not less than two days after collection, the pupae were separated from the bran, either by sieving or in an air stream,

and then stored in screen-bottomed trays. To minimize a heat build-up, the depth of pupae in the tray never exceeded 2 cm.

To obtain pupae of as near as possible the same biological age, each day's collection of pupae was stored at temperatures of 20°, 25° or 28°C for varying periods (Nadel and Guerrieri (1969)).

Marking of pupae

On the day of shipment, pupae from the various holding trays were mixed and then marked with ultra-violet fluorescent powders. Approximately one gramme of powder adequately marked 20 000 pupae, and 100 000 pupae were marked at a time. Four fluorescent colours (green, red, orange and yellow) were used in rotation, one colour for all of one week's consignment, so that when irradiated flies were trapped in the field not only could the time of release be determined but the ratio of irradiated to wild flies could be established.

Irradiation of pupae

To obtain sterile male flies whose sexual competitiveness is optimal, irradiation of pupae should be carried out within the last 24 hours of pupal life (Katiyar et al. (1966)). However, because the pupae did not arrive on Procida until 24 hours after irradiation, and in order to avoid a large emergence of flies during this period, the pupae were irradiated 48-24 hours before adult emergence was expected. Thus, by the very nature of the program, the most competitive males were not available for release. The biological age of the pupae was established by the eye-colour of the pharate fly within the puparium. Over the last three days of pupal life the colour of the eyes changes from light yellow through pink, dark red and finally to blue. The actual timing of these changes relative to adult emergence will depend on the temperature at which the pupae are stored.

Pupae were given a centre field dose of 9 krad in a ^{60}Co gamma irradiator (Gammacell 220). Only 0.9 litre of the 3-litre-capacity irradiation chamber was used to reduce the variance in dose associated with the geometry of the chamber to ± 11%. The dose rate at the mid-point of the irradiation chamber (determined by Fricke dosimetry) decreased from 9.2 to 9.0 krad/min during the experiment.

During a calibration of the dose rate it was found that while a vial of Fricke solution placed at the bottom of the irradiation chamber received 8.4 krad/min, when surrounded by a 4-cm ring of mature pupae the dose rate dropped to 7.9 krad/min i.e. by almost 6%. This degree of absorption of gamma radiation by pupae was unexpected and as a result the variance in dose received by the experimental pupae would have been greater than ± 11%.

Pupae were irradiated in an open-topped polythene container which held 0.9 litre of pupae (depending on the size of the pupae, this volume contained 40 000 - 55 000 pupae). Pupae were transferred to the irradiation container just before treatment to avoid any effect of anoxia. However, in a limited test, pupae were stored for 30 min in the polythene container and then irradiated. Male flies which emerged from pupae at the base of the container, where any effect of anoxia would have been

most severe, showed normal levels of sterility. It was concluded that anoxia was no problem during the routine irradiations when pupae were in the irradiation container for no more than 3 - 4 min.

To minimize the possibility of shipping a batch of unirradiated pupae, the irradiations were normally carried out by one of the authors, while two technicians handled all other associated tasks. Towards the end of the program glass ampoules of a radiosensitive solution which underwent a colour change when exposed to gamma radiation were used (Moos et al. (1970)).[1] No discernible change in the red colour of the radiosensitive solution occurred with less than 1 krad which would result if the timer on the Gammacell was not reset. The desired dose of 9 krad produced an obvious colour change (from red to pink) and after 18 krad (which would result if a batch of pupae was irradiated twice) the solution was colourless. The dosimeter was simple to use, had a shelf-life of 4 - 8 weeks if stored in the dark at normal room temperatures and, if prepared in large numbers, would be relatively inexpensive (1 - 2 US cents/ampoule). It warrants use in future similar programs.

Packaging and shipment of pupae

Immediately after irradiation the pupae were placed in shallow cardboard trays (39.5 × 5 × 1 cm) which were covered with tight-fitting terylene sleeves. Each tray held approximately 0.9 litre pupae. The open end of the cloth sleeve was securely closed to prevent escape of any flies which emerged during transport. The trays of pupae were placed in two vertical tiers in a wooden-framed open-sided shipping container (dimensions: 43 × 34 × 43 cm high, Fig.1). The container was an improvement over those used previously in that an additional vertical support was provided in the centre of the container which prevented the cardboard pupal trays from collapsing. The trays were separated by 1 cm spacers to permit aeration and dissipation of metabolic heat. A shipping container held approximately 19.8 litres of pupae (792 000 to 1 089 000 pupae) in 22 trays, and fully loaded weighed approximately 15 kg. The pupal consignment was transported from Vienna to Rome in the pressure- and temperature-controlled passenger section of a commercial aircraft. Normal flight time was 1.45 hours. The number of pupae shipped during the experiment is shown in Table I.

3. QUALITY CONTROL TESTS

During the 1968 Procida experiment, it was found that the percent emergence of flies varied widely between consignments. It was concluded that this was due to the various pupal handling procedures and that the irradiation treatment was not a contributing factor (IAEA (1969)). For the 1969 experiment, tests were implemented to routinely assess the emergence of flies from treated pupae and the sterility of the irradiated males. Pupal samples for these tests were taken after the pupae were irradiated and just before packaging.

---

[1] The dosimeter was developed and provided by W.S. Moos, J. Nagl and J. Haider of the Dosimetry Section and Dosimetry Laboratory, IAEA, Vienna.
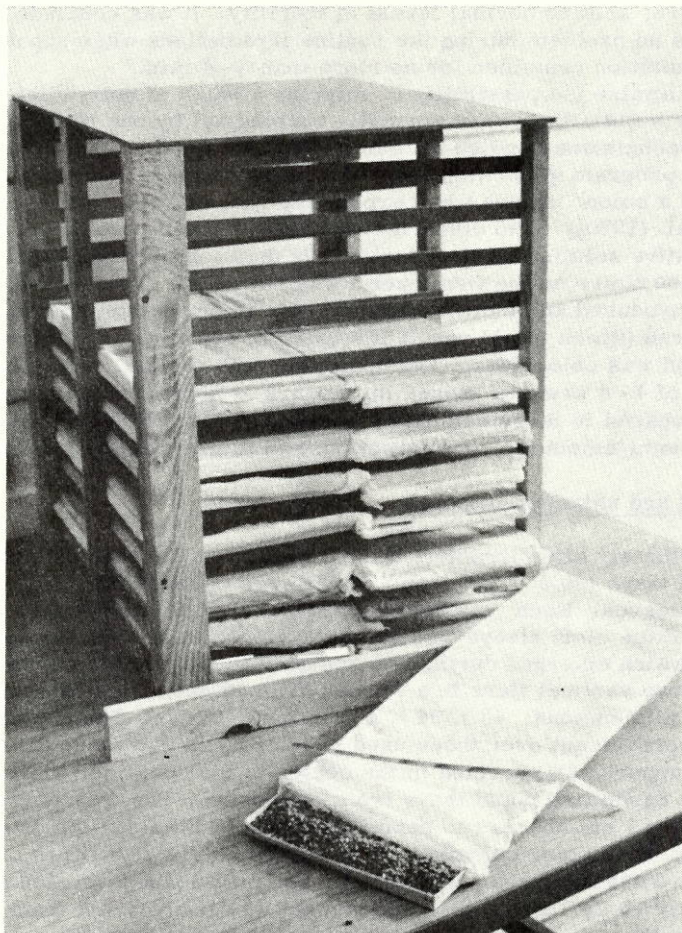
FIG. 1.   The shipping container used to transport mature pupae. Note the method of packaging the pupae and the use of spacers to separate individual trays of pupae.

Adult emergence

Samples of approximately 150 pupae were taken from each of 20 randomly selected shipping trays per consignment. These samples were kept individually and the percent adult emergence three days after irradiation and the total emergence were recorded. The 20 sampled trays were identified and numbered and these were sampled again by CNEN staff upon arrival on Procida.

The total emergence (Table I) estimates the viability of the pupae. This viability is the result not only of the larval rearing procedure but also of the treatment and handling of the pupae. The mean emergence per consignment fluctuated rather widely and more than was acceptable.

TABLE I. NUMBER OF IRRADIATED PUPAE SHIPPED TO ITALY,
AND ADULT EMERGENCE AND MALE STERILITY DATA FOR EACH
SHIPMENT

Shipment No.	Date irradiated and shipped	No. pupae shipped (in millions)	% Adult emergence [a]		Male sterility [b] % egg hatch
			After 72 hours	Total	
1	18.3.69	0.3	89 (83-97)	91 (83-100)	5.3 (640)
2	25.3.	0.9	68 (44-84)	75 (61-84)	5.5 (794)
3	1.4.	1.4	74 (58-89)	74 (58-89)	4.1 (2007)
4	9.4.	2.2	65 (52-81)	66 (53-84)	1.4 (1484)
5	16.4.	0.5	62 (54-70)	70 (62-77)	-
6	23.4.	0.3	81 (77-83)	81 (78-84)	-
7	28.4.	0.3	74 (72-77)	74 (72-77)	-
8	5.5.	2.1	63 (43-88)	67 (48-88)	3.2 (2647)
9	15.5.	1.6	69 (56-90)	82 (71-90)	1.0 (101)
10	19.5.	0.8	73 (65-78)	-	1.0 (3726)
11	21.5.	1.0	92 (89-96)	92 (89-96)	0.9 (3159)
12	27.5.	3.2	88 (75-93)	88 (78-93)	2.6 (2571)
13	3.6.	2.3	49 (35-66)	-	4.4 (1523)
14	10.6.	1.4	52 (37-70)	-	1.9 (1954)
15	19.6.	3.1	54 (48-60)	77 (70-86)	2.4 (2942)
16	24.6.	1.7	41 (31-58)	67 (53-87)	4.2 (2780)
17	1.7.	1.2	51 (37-62)	90 (82-94)	1.7 (2741)
18	3.7.	1.5	All pupae died		
19	8.7.	1.0	18 (3-58)	88 (82-94)	1.5 (2902)
20	10.7.	0.6	73 (55-79)	80 (70-86)	0.7 (1803)
21	15.7.	3.1	47 (40-58)	72 (62-79)	1.2 (2730)
22	22.7.	1.0	69 (62-80)	91 (88-94)	1.6 (3276)
23	31.7.	1.5	71 (62-80)	75 (64-81)	1.2 (3304)
24	5.8.	3.0	73 (64-79)	88 (82-93)	2.6 (3255)
25	12.8.	2.0	68 (54-79)	86 (78-91)	3.2 (1607)
26	19.8.	2.0	22 (3-36)	91 (86-95)	2.9 (3302)
27	26.8.	1.6	28 (1-49)	78 (66-84)	1.9 (1616)

[a] Figures in parenthesis indicate the range of emergence.
[b] Hatch of eggs obtained from normal females mated with irradiated males; figures in parenthesis indicate the number of eggs on which the percent hatch was based.

We believe that much of this variation was due to the need to retard or increase the rate of pupal development. The occasional wide variation in emergence between shipping trays within a consignment (e.g. data for shipment 8), which is indicated by the range values in parenthesis, was undoubtedly due to inadequate mixing of the various pupal lots before irradiation.

In the 1969 experiment it was believed that only those flies which emerged within 3 days of irradiation would have an adequate level of competitiveness. It was for this reason that the emergence after 3 days was measured. The variable 3-day emergence data (Table 1) indicate that on a number of occasions pupae were not irradiated at the desired stage of pupal development. This can be attributed almost entirely to the need for controlling the rate of pupal development.

When the 3-day emergence data from Seibersdorf and Procida were compared (for the same shipping trays) it was clear that emergence was usually, and often appreciably, lower on Procida. This must be attributed primarily to adverse conditions experienced by pupae en route from Seibersdorf to Procida. Sampling error would also contribute to variations in emergence recorded at Seibersdorf and Procida, and this point was investigated. In one experiment 19 samples of approximately 170 pupae were taken from one tray of pupae immediately before irradiation. After irradiation the tray of pupae was kept at 25°C for 24 hours (equivalent to the time elapsing from departure of pupae from Seibersdorf to arrival on Procida) when 19 further samples were taken. The percent emergence of flies, three days after irradiation, was recorded for both sets of samples. The results (Table II) show that in the absence of irradiation a considerable variation in emergence can be expected, due solely to sampling errors. The magnitude of this variation decreased when samples were taken after irradiation (the coefficients of variation were 14.4% and 9.8% respectively).

A further experiment was carried out with samples from one tray of pupae taken immediately before and immediately after irradiation and then 24 hours later. The results (Table III) confirmed those obtained in the previous experiment. In both experiments the data clearly show that the irradiation treatment accelerated the emergence of adults.

TABLE II. VARIATION IN PERCENT EMERGENCE OF ADULT FLIES DUE TO SAMPLING ERROR; SAMPLES FOR EMERGENCE WERE TAKEN FROM THE SAME BATCH OF PUPAE

	Sampled before irradiation	Sampled 24 hours after irradiation
No. of samples	19	19
Mean no. of pupae per sample	171.5	159.6
Mean emergence (%)	66.4	80.5
Range of emergence (%)	42.1 - 85.8	53.8 - 95.4
95% CL of mean (%)	46.3 - 86.5	64.0 - 97.0

TABLE III. VARIATION IN PERCENT EMERGENCE OF ADULT FLIES
DUE TO SAMPLING ERROR; SAMPLES FOR EMERGENCE WERE
TAKEN FROM THE SAME BATCH OF PUPAE (second experiment)

	Sampled before irradiation	Sampled immediately after irradiation	Sampled 24 hours after irradiation
No. of samples	17	17	17
Mean no. of pupae per sample	202.3	212.2	205.6
Mean emergence (%)	79.2	88.6	89.9
Range of emergence (%)	66.8 - 90.6	84.1 - 93.3	85.3 - 93.8
95% CL of mean (%)	65.1 - 93.3	83.3 - 93.9	85.3 - 94.5

Male sterility

A further sample of approximately 150 pupae was taken from each
of the 20 randomly selected shipping trays mentioned earlier. These
samples were combined. From the flies that emerged over a 24-hour
period, males were selected and mated with normal females. Four
cages, each containing 25 irradiated males and 25 normal females,
were established for each pupal consignment. Samples of approximately
200 eggs were taken from each cage twice per week for 2 weeks and
egg hatch was recorded after 5 days at 25°C. From shipment 19 on,
the procedure was changed to conform with that carried out at the
CNEN Laboratory. A sample of approximately 100 pupae was taken
from every shipping bag and the males which emerged from the pooled
samples were used for the tests.

Overall, egg hatch was acceptable and within the limits to be ex-
pected from a dose of 9 krad ± 11% (Table I). While in precise dose-
sterility experiments we have obtained a mean egg hatch of 1.9% with
a dose of 9 krad, within one replicate the day-to-day egg hatch data
can vary from 0.8% to 6.9% (Hooper, unpublished data). Therefore,
the occasional values of 3 - 5% hatch obtained during this program are
not indicative of any deficiency in the irradiation procedure.

4. DISCUSSION

Since it was necessary that the main emergence of flies from the
irradiated pupae did not commence during the 24 hours or more which
elapsed between irradiation at Seibersdorf and arrival on Procida, the
most competitive males were precluded from the experiment. This
point should be remembered when examining the results of the field
experiment (de Murtas et al. (1970)).

The quality control tests indicated that:

(a) there was inadequate mixing of the various batches of pupae
     before irradiation,

(b) all pupae were not in the same developmental stage when irradiated, and

(c) total emergence of flies was below the desired level on a number of occasions.

The need to adjust the rate of development of the pupae, in order to ship a maximum number on a once-per-week schedule, contributed to a greater or lesser degree to each of the above problems. If it had been possible to release sterile flies several times per week, problems (a) and (b) would have been eliminated and the severity of problem (c) reduced. Therefore, in any future suppression or eradication experiment, multiple releases per week should be considered a primary requirement. If for any reason the rearing facility must be located at a considerable distance from the release area, then young pupae should be shipped and irradiated close to the release area (quarantine regulations permitting).

The chemical dosimeter described and used in part of the program is worthy of further testing as a simple check on irradiation procedures.

REFERENCES

IAEA (1965). Advances in Insect Population Control by the Sterile-Male Technique, Tech. Rep. Ser. No.44, IAEA, Vienna.

IAEA (1968). IAEA Laboratory Activities, Fifth Report, Tech. Rep. Ser. No.90, IAEA, Vienna, p.64.

IAEA (1969). IAEA Laboratory Activities, Sixth Report, Tech. Rep. Ser. No.98, IAEA, Vienna, p.57.

KATIYAR, K.P., FERRER, F., VALERIO, J. (1966). The Application of Nuclear Energy to Agriculture, Triennial Report, July 1963 - June 1966, of the Inter-American Institute of Agricultural Sciences of the OAS, Turrialba, Costa Rica, p.45.

MOOS, W.S., NAGL, J., HAIDER, J. (1970). A simple and inexpensive chemical radiation dose indicator, Int. J. appl. Radiat. Isotopes 21, pp.177-178.

MURTAS, I.D. de, CIRIO, U., GUERRIERI, G., ENKERLIN-S., D., An experiment to control the Mediterranean fruit fly on the island of Procida by the sterile-insect technique, these Proceedings.

NADEL, D.J., GUERRIERI, G. (1969). "Experiments on Mediterranean fruit fly control with the sterile-male technique", Sterile Male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968) IAEA, Vienna, p.97.

NADEL, D.J., PELEG, B.A. (1966). "Mass rearing techniques for the Mediterranean fruit fly in Israel", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests (Proc. Panel Tel Aviv, 1966) IAEA, Vienna, p.87.

AN EXPERIMENT TO CONTROL
THE MEDITERRANEAN FRUIT FLY
ON THE ISLAND OF PROCIDA
BY THE STERILE-INSECT TECHNIQUE*

I.D. de MURTAS, U. CIRIO, G. GUERRIERI
Comitato Nazionale per l'Energia Nucleare,
Rome, Italy
and
D. ENKERLIN S.†
Joint FAO/IAEA Division of Atomic Energy
in Food and Agriculture,
International Atomic Energy Agency,
Vienna

Abstract

AN EXPERIMENT TO CONTROL THE MEDITERRANEAN FRUIT FLY ON THE ISLAND OF PROCIDA BY THE STERILE-INSECT TECHNIQUE.

During 1969 an experiment to evaluate the sterile-insect technique as a method of control for the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), was conducted on the island of Procida. Although releases were initiated rather late (16 May) and with fewer flies than originally planned, adequate protection of most of the commercial peaches and apricots was achieved. In the release area a number of fruits showed punctures without eggs. These are considered as mechanical damage done by released sterile females but the economic importance of such punctures could not be determined. Observations indicate that at least part of the Mediterranean fruit fly population overwinters in sour orange varieties. These fruits should be picked during the winter and destroyed, thus at least reducing the new population in the spring.

INTRODUCTION

The damage caused by the Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), to Italian agriculture is increasing mainly because farmers are finding it harder to choose suitable means of control in view of the difficulties frequently associated with the use of insecticides, such as undesirable residues and biological disequilibrium. The sterile-insect technique has opened up new possibilities of insect pest control.

The originator of the sterile-male technique, Knipling (1955), formulated its basic theory as long ago as 1938, although the method was not applied in the field until 1954 (Baumhover et al. (1955), Knipling (1960)). Fruit flies (Ceratitis, Dacus and Anastrepha) were the object of the first investigations in the field of agricultural entomology. Steiner and co-workers (1952, 1956a, b) demonstrated that practical control could be obtained by releasing sterile fruit flies into the native population.

In 1967, under the auspices of the IAEA, two successful experiments for controlling the medfly were carried out using the sterile-male technique. One, reported by Rhode (1970), was in Nicaragua, Central America; the other, reported by Nadel and Guerrieri(1969) was on Capri, Italy.

In 1968 and 1969 the island of Procida was chosen by scientists of CNEN, IAEA and MAF[1] as the area for further research work because of its special agricultural characteristics, i.e. it contained many different hosts of Ceratitis. The main objectives were:

   (1) In 1968, to collect bioecological background information, on wild, normal and released sterile flies, which would permit a rational application of the technique (de Murtas, Cirio and Enkerlin (in press)).

   (2) In 1969, (a) to establish whether it would be possible to use the technique for the control or eradication of Ceratitis, and
       (b) to determine whether the sterile flies, released in large numbers, could cause any damage to the fruit.

The program was initiated in April 1968 with a series of bioecological observations comprising studies of population, movement, capture and hosts. At the same time various systems of releasing irradiated flies were compared with reference to the radiation dose applied, the stage of the insects treated and the feeding prior to release. The results of this phase of the work are reported by de Murtas, Cirio and Enkerlin (in press).

For the 1969 season, a full-scale control experiment with weekly releases of several million flies was planned. Results of this experiment are discussed in the present paper.

MATERIALS AND METHODS

A. Description of the environment

(1) Release area

Procida, the smallest of the Parthenopean islands, has an area of 3.7 km^2, is flat and elongated and characterized by intensive cultivation of citrus and grapes, frequently associated with peaches, medlars, figs and apricots. A few plants of the Opuntia genus, carob trees, olive trees, generally isolated, are to be found in the uncultivated zone near the sea. The favourable climate and environment and the extremely fertile land of volcanic origin allowed a fairly dense human population to settle, splitting up the island into over a thousand holdings, used for fruit growing, mainly on a family basis. These factors have provided Ceratitis with ideal conditions for causing serious damage.

(2) Control areas

(a) The surroundings of Bacoli, not far from the mythical Cape Miseno, face directly on a channel, a few miles wide, which separates the islands of Ischia and Procida from the mainland. Broadly speaking, the orchards

---

[1] Ministry of Agriculture and Forestry, Italy.

here follow the typical pattern of general fruit growing which has developed on a family basis. In contrast to the inhabitants of Procida, the farmers of the region rely very frequently on the use of agricultural chemicals to control infestations of the fruit fly, which, as on Procida, are found in very large numbers.

(b) Capri is the best known of the Parthenopean islands. Its special geographical conformation, its relative isolation from the mainland and, last but not least, the bioecological information gathered during and after the 1967 experiment, convinced us that it would be a good idea to carry out the control observations also on this island.

(3) Meteorological data

On Procida the meteorological data were collected in the immediate vicinity of the laboratory where a small hut was fully equipped with a hygrothermograph, a rain gauge and an anemometer. Two other hygro-thermographs were installed, one near Marina-Porto to the north and one at Punta Solchiaro, in the south of the island, which is characterized by a climate contrasting with that of the other areas of Procida.

The data on the island of Capri were provided by the Air Force Meteorological Service.

B. Irradiated insects

The release of sterile flies in the field commenced on 17 May 1969. On the basis of the tests carried out in the preceding month on the northern slope of the island it was considered appropriate to release two-day-old fasting Ceratitis adults obtained from pupae irradiated at a dose of 9000 rad with a cobalt-60 source and marked with suitable fluorescent dyes.

The rearing of the flies, marking and gamma irradiation were carried out at the Seibersdorf laboratory of the IAEA (Nadel and Guerrieri (1969)). The pupae, irradiated 1 – 2 days before emergence, were sent by air from Vienna to Rome in special well-aerated containers, which completely eliminated any risks to the insects from a possible temperature increase produced by their intense metabolic activity.

On arrival, the pupae were immediately transported in an air-conditioned vehicle at a temperature of 25°C to Pozzuoli, from where they were taken by boat to Procida and a rustic laboratory located in a house in an isolated area. There they were put in special 50 cm × 40 cm paper bags, each containing approximately 5000 specimens. These bags were placed in a closed area in the cellar with a fairly constant temperature of about 24°C. When shipments arrived on the island, about 20 samples of approximately 200 pupae were taken from trays which had been marked for this purpose at the Seibersdorf laboratory. Each sample was placed in a Petri dish in order to obtain percent emergence data 72 hours after the arrival of the shipment. In addition, daily observations of release bags and Petri dishes were made in order to determine the day on which flies should be released and to determine percent emergence as compared with emergence in the Seibersdorf laboratory (Hooper (1970). However, flies were never kept in the laboratory longer than three days.

TABLE I. DATA ON RELEASE AND RECAPTURE OF MEDFLY DURING THE 1969 EXPERIMENT ON THE ISLAND OF PROCIDA, ITALY

Date of arrival[a]	No. pupae per shipment (in millions)	Percentage emergence[b]	Adults released (in millions)	Trapping date	No. of captured males		Ratio
					marked (sterile)	unmarked	sterile: wild
16/5	1.60	62	0.99	15/5	64	10	-
19/5	0.80	-	-	-	-	-	-
21/5	1.00	95	0.95	23/5	1952	14	139:1
27/5	3.20	80	2.24	-	-	-	-
3/6	2.30	55	1.26	5/6	3678	1	3776:1
10/6	1.40	51	0.71	12/6	4347	103	42:1
17/6	3.10	46	1.43	19/6	2500	77	32:1
24/6	1.70	64	1.09	26/6	4454	139	32:1
1/7	1.20	67	0.80	3/7	4363	152	29:1
3/7	1.50	-	-	-	-	-	-
8/7	1.00	75	0.75	10/7	2636	100	26:1
10/7	0.80	77	0.46	-	-	-	-
15/7	3.10	71	2.20	16/7	3579	56	64:1
23/7	1.00	81	0.81	24/7	1716	342	5:1
31/7	Not received	-	-	29/7	580	270	2:1
6/8	3.00	30	0.90	6/8	225	241	1:1
13/8	2.00	86	1.70	-	-	-	-
19/8	Not received	-	-	20/8	2695	323	12:1

a Flies were released when a high percentage had emerged, but never later than 72 hours after arrival
b Taken in Petri dishes

C. Release of the Irradiated Insects

Before proceeding to the first release of sterile flies, a large number of bioecological observations were conducted and population (de Murtas, Cirio and Enkerlin, in press), flight distance (de Murtas, Enkerlin and Cirio, in press), host and predator studies (Cirio, personal communication to Boller) were performed. Particular care was taken in distinguishing the preferential hosts. On the basis of the results obtained, the island was split up into 426 zones containing release situations 50 – 100 m from each other. At the points of maximum host-plant density some additional releases were carried out. Previously, individual release stations had been selected and marked by tying a coloured cloth strip to the branch of a suitable tree. Each week the bag containing the irradiated flies was attached to the cloth on a branch and ripped open, or if a particular garden was closed, the bag was thrown, opened, over the wall.

The numbers of adults released are given in Table I. It was hoped to release 3 – 4 million flies per week in order to overflood the native population with about 10 thousand sterile flies per hectare per week, but owing to technical problems with the rearing in the Seibersdorf Laboratory and the rather low percentage emergence on the island, weekly releases were much lower.

D. Methods for evaluating the effectiveness of the technique

Continuous control was needed in order to obtain precise information on how the experiment was proceeding. With this purpose in mind, use was made of direct methods such as examination of the fruit to check for infestation and obtaining eggs to determine percentage egg hatch, and of an indirect method by weekly trapping of flies.

(1) Examination of the fruit

The purpose of examining the fruit was to make a quantitative comparison of the degree of infestation of the fruit in the experimental area and the control areas. For this purpose we selected on Procida 25 representative orchards characterized either by the presence of several hosts or by different conditions broadly representative of most of the microhabitat of the island. Because of heavy rains in the spring, the main flowering period of apricot and peach trees, the fruit of these two main hosts was quite scarce and farmers in most areas could not be persuaded to sell ripening fruit to the research workers. Therefore, infestation data were taken in two ways. The first consisted of manual and visual examination of the fruit hanging on pre-determined trees selected at random. This method of inspection is not considered to be very accurate. The second consisted in picking at random an amount of fruit from the same trees and in placing it in suitable boxes over a metallic mesh, which allowed any larvae present to develop and fall to the bottom. It was lined with sawdust to facilitate pupation (Fig. 1). A similar procedure was followed in the case of the fruit gathered in the control areas. This very accurate method could be done only occasionally because of the very limited amount of fruit.

FIG.1.   Rudimentary box to keep fruit for later examination.

(2)   Determination of percentage egg hatch

One of the major problems in the field for those employing the sterile-male technique is to determine the extent of matings of irradiated males with normal females, which is the indication of the effectiveness of the released insects. Determination of egg hatch in our experiment provided an excellent solution to this problem.

Peaches and apricots provided excellent material for these studies. Citrus fruits were less suitable because the skin easily masks the puncture made by Ceratitis and the oviposition chamber hardens completely.

The procedure was as follows. In the orchards mentioned on Procida and in the control area at Bacoli, fruit was gathered at random periodically and taken to the laboratory. Each fruit was carefully examined. First of all the punctures were counted and then at each puncture a circular section was removed with a razor blade and subsequently cut open to reveal the oviposition chamber. Any eggs or the chorion shed by the larvae were collected with a fine, hard, nylon thread under a stereoscope. Unhatched eggs were placed for three or four days on humid filter paper in Petri dishes. The results are given in Table III.

(3)   Trapping method

An indirect method of evaluating the effectiveness of the technique is to capture flies in the field and determine the ratio of marked (sterile) to unmarked (fertile) flies. For this evaluation special plastic traps of the 'Nadel type' were used; four were set in each of the 25 representative orchards or group of gardens on Procida in peach, medlar, fig and lemon

trees respectively. The distance between the traps in each area was approximately 75 m. The bait used was a mixture of trimedlure and 1% DVVP.

The traps were placed once a week in previously marked trees for 24 hours, usually the day before the subsequent release, and were collected shortly before that release. The captured adults were placed in the dark on a sheet of black paper and examined under an ultra-violet light (254 mu/220 V bulb) which allowed the colour-marked (sterile) flies to be distinguished immediately from the unmarked insects. For greater reassurance the heads of apparently unmarked flies were crushed and examined, by means of a stereoscope, for traces of the dye on the ptilinum.

EXPERIMENTAL DATA AND DISCUSSION

(a) Infestation of the fruit

Table II shows the percentages of infested fruit on Procida and in the control areas for each series of observations carried out between 30 April and 30 August. The data on the fruit inspections in the period from September to December are not given, since the last release of sterile flies took place on 13 August and the experiment was not aimed at eradication.

On Procida it was found that the common oranges suffered practically no damage from Ceratitis. Of the 3735 oranges examined, only one had been attacked. The situation with respect to pears was similar in all cases. In peaches and apricots infestation was reduced to a level which the farmers described as negligible after being resigned for decades to the loss of more than half of their harvest every year. The maximum percentage of infested fruit was 4.8 for apricots in mid-July and 7.6 for peaches in mid-August. During the previous year 16% of the apricots had been infested at the same time and 92% of the peaches.

Sour oranges deserve special comment. This fruit, which is not edible for man, underwent strong attack from medfly. Apparently, during the winter and spring, sour oranges are preferred to common oranges. On the other hand, the latter usually fall to the ground once infested, whereas sour oranges stay for a long time even if attacked by medfly; this makes them the ideal overwintering host. Even heavy release of sterile flies close to sour orange trees did not reduce infestation. The spread of fertile females to other areas was apparently prevented. It is concluded from this that if the medfly can vary its behaviour in its choice of host, sour oranges, which are not normally harvested, might be preferred. Particular attention should, therefore, be devoted to this fruit as on Procida it provides centres of infestation.

The data on the control areas present quite a different picture. In the orchards of Bacoli-Capelle it was found that attack on the fruit got out of control, as shown by the extremely high percentage of infestation in peaches, apricots and pears, notwithstanding the fact that farmers had treated the trees no less than four times with insecticides based on Rogor and Parathion in June and July.

On Capri, fruit infestation by the medfly began later than on Procida and at Bacoli, but by the end of July apricots were totally infested, while in the period 15-30 August, damage to peaches increased from 3.2 to 82.9%.

TABLE II.　NUMBER OF DIFFERENT HOST FRUIT EXAMINED AND PERCENTAGE ATTACKED BY MEDFLY IN THE RELEASE AND CONTROL AREAS

Date of collection	LOCALITY[a]									
	Procida					Bacoli[b]			Capri	
	Oranges	Sour Oranges[c]	Peaches	Apricots	Pears	Peaches	Apricots	Pears	Peaches	Apricots
30/4	1270 0.0	211 1.9	- -	- -	- -	- -	- -	- -	- -	- -
15/5	1298 0.0	668 3.1	- -	- -	- -	- -	- -	- -	- -	- -
30/5	496 0.0	227 0.4	232 0.0	- -	- -	- -	- -	- -	25 0.0	- -
15/6	441 0.2	928 14.3	1674 0.0	447 0.0	- -	100 0.0	- -	- -	246 0.0	- -
30/6	170 0.0	606 15.2	2285 0.2	1092 0.3	- -	197 1.8	47 10.6	- -	168 0.0	78 0.0
15/7	50 0.0	81 68.7	1476 0.5	857 4.8	450 0.0	210 25.7	168 41.1	- -	- -	324 16.0
30/7	10 0.0	31 80.6	874 6.0	15 0.0	90 1.1	175 80.6	250 84.0	287 38.3	- -	100 10.0[d]
15/8	- -	117 92.3	1784 7.6	- -	310 0.0	- -	- -	- -	1193 3.2	- -
30/8	- -	31 67.7	2213 7.2	- -	560 0.5	30 93.3	- -	500 83.0	1002 82.9	- -

a　First column indicates fruit examined; second column refers to percentage infested
b　June and July data in spite of 4 pesticide treatments
c　On last 4 dates fruit was picked in two gardens only
d　Fruits collected from the ground

TABLE III. DATA ON PUNCTURES AND EGG FERTILITY PER
100 PEACHES IN THE RELEASE AND CONTROL AREAS
(PUNCTURED PEACHES SELECTED FOR STUDY REPRESENTING
NON-RANDOM SAMPLE)

| Criteria | Locality | | % reduction |
	Procida	Bacoli	
Total no. punctures	197.4	236.5	-
Punctures without eggs	81.1	0.2	-
Punctures with eggshells (hatched eggs)	40.7	226.5	82.0
No. eggshells	264.5	1809.1	85.4
Punctures with eggs	68.3	9.2	-
No. eggs (placed in Petri dishes)	658.2	68.4	-
% egg hatch in Petri dishes	4.0	64.2	-
Punctures with eggs and shells	109.0	235.7	53.8
Total hatched eggs	293.1	1853.0	84.2
Total percentage hatch	31.4	93.2	-

(b) Results of fruit examination

Table III presents data on numbers of punctures, number of eggs and
percentage fertility when collecting 189 apparently stung ripening peaches
on Procida and 98 in the control area (Bacoli-Capelle). Data were trans-
formed for 100 fruit. A much larger sample of stung fruit would have been
more meaningful, but could not be obtained for several reasons. The total
number of punctures was only somewhat higher in the control area but most
of the punctures had hatched eggs (empty chorions or egg shells). On
Procida the highest number of punctures (83.1) had no eggs indicating that
they were made by released radiated females. The number of punctures
with hatched eggs (fruit with larvae) was over 82% less in the release area
than on the mainland.

As expected, many more punctures (68.3) with unhatched eggs (658) per
100 peaches were found in fruits from Procida as compared with the control
area (9.2 punctures with 68 eggs per 100 peaches). When placing these
eggs on filter paper in Petri dishes only 4% of those from the release area
hatched as compared with 64.2% of eggs from the control area (Bacoli).
The considerable number of sterile eggs found in oviposition chambers
confirms a substantial number of matings between sterile males and normal
females. From fruit picked in the release area 40.7 punctures revealed
empty eggshells. In all cases, when these peaches were carefully opened,
larvae could be found close to the stone. Therefore, the number of empty
eggshells are expressed as hatched eggs. By adding these to the eggs
hatched in Petri dishes, the total number of eggs hatched per 100 stung
fruit in the release area was found to be 293.7 as compared with 1853.1

in the control area; this means a possible 84.2% reduction in young larvae. All data were taken in medium to late varieties during the latter part of July. During this month the ratio of sterile flies to normal flies dropped rapidly and infestation was expected. In the early varieties reduction of infestation was much higher. Punctures of fertile females could not be found and farmers reported the best fruit in many years.

(c) Release data and trapping observations

The first columns of Table I show the number of pupae shipped to Procida during the release period, the percentage emergence and number of adults released. When comparing the percentage adult emergence on the island of Procida and in the Seibersdorf laboratory (Hooper and Nadel (1970)), it can be seen that emergence was not detrimentally affected by the shipping procedure except in late June and on 6 August. There could be several reasons for poor emergence on these dates; for example, heat exposure at the Rome airport or during the transport from Rome to Procida. On several occasions emergence at 72 hours was higher on the island than in the Seibersdorf laboratory.

In the last columns of Table I, trapping data during the 1969 release operation are shown. It can be assumed that marking efficiency was as good as possible and probably in the vicinity of 99.6% as reported by Rhode (1970), when using a similar method of dyeing and observing the captured flies by means of u.v. light. We therefore assume that the unmarked flies in all cases except on 5 June were wild flies and their number is apparently in relation to the number of sterile flies released and the increase of the wild population as the season advanced. The sterile-to-wild ratio dropped considerably after mid-June and this correlates with the increase of fruit infestation (Table II).

In Fig. 2 the number of wild males captured per trap, per day, are presented for the 1968 and 1969 spring and summer months. It should be noted that in 1969, when sterile flies were being released weekly, the population of wild males remained very low as compared to 1968. It increased after August, but releases of sterile flies were stopped in mid-August.

CONCLUSIONS

The use of sterile insects for the control of insect pest populations undoubtedly marks a new chapter in the field of applied entomology. This technique, which is among the most sophisticated of the biological control systems, in our opinion fits perfectly into the wider context of integrated control, provided that due attention is paid to all the intrinsic biological factors concerning the insect and that more detailed studies are made of the limiting factors to which the insect is subject when released. This entails in the first place the solving of complex problems, such as mass rearing of the insect, irradiation with the right dose, and transport, in order to have the most competitive flies possible. In the second place, farmers have to be convinced of the effectiveness of the sterile-insect technique so that all agree to its application at the most convenient moment in order to achieve success.
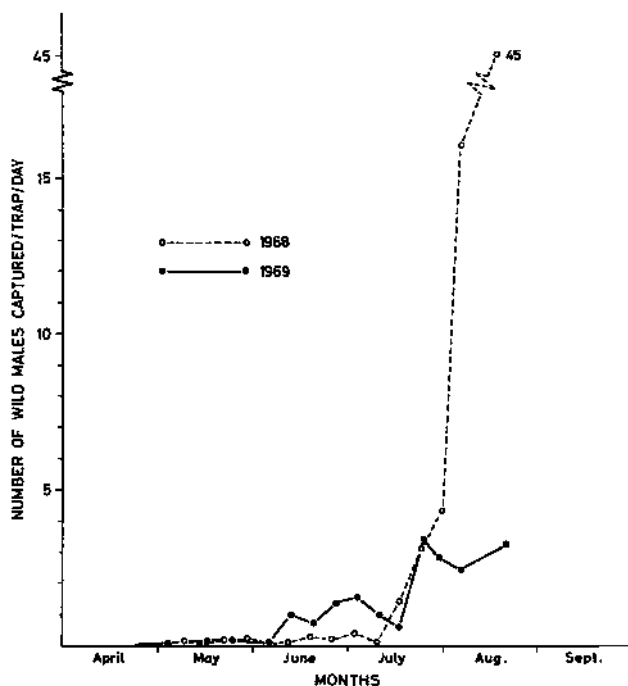
FIG.2. Number of captured wild males on Procida.

The manual release of the irradiated adults, while undeniably having the advantage of making cautious operation possible, caused an extra burden of work because of its slowness and because of the difficulty in finding sufficient technical labour. It is therefore to be hoped that future releases can be made from the air. The test conducted by Nadel et al. (1967) with aircraft (or helicopters) and by de Murtas (unpublished data) with a helicopter, and the large-scale experiment in Central America (Rhode (1970)) gives grounds for such hopes.

A third point, no less important than mass rearing of the species under study, has been the collection of all the information on the behaviour of the insect in the field, which has enabled us to determine that releases on Procida should start as early as late March to reduce to a minimum the number of sterile flies required to obtain a maximum degree of control. In this connection the value has been proven of trimedlure traps, used not only for the study of the population dynamics but also for observing the dilution and movement of the species in the surrounding environment.

The data given in the tables indicate that the irradiated insects have carried out their task. Meanwhile, the numerous unhatched eggs found in fruit provide a clear answer to our main question, namely, whether it is possible to apply the sterile-insect technique successfully.

Moreover, the reduction of the fertile population on the island, and the considerable reduction in the damage to fruit – despite the fact that the male sterile-to-fertile ratio was not adequately maintained because of the

brief period of the experiment, and despite late initiation of release and insufficient numbers, due to logistic and personnel problems – has led us to conclude that the sterile-male technique when skilfully employed is a tool which will continue to find increasing application for the control of insect pests, especially fruit flies. We believe this all the more when we consider that the technique, although complicated and rather expensive, appears to hold no dangers for man.

The sterile female medfly, when released in great numbers, causes mechanical damage through punctures without eggs. The economic significance of this damage to peach and apricot remains to be determined. Sour orange varieties are adequate hosts and at least part of the medfly population on the island spends the winter in this fruit as grown larvae. So it is recommended that farmers pick these fruits during late winter and destroy them, thus reducing the overwintering population.

REFERENCES

BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, D.E., NEW, W.D., DUDLEY, F.H., BUSHLAND, R.C. (1955) Screwworm control through release of sterilized flies, J. econ. Ent. 48 (4), pp.462-466.

DE MURTAS, CIRIO, U., ENKERLIN-S., D. (in press), Dispersion de Ceratitis capitata (Wiedemann) dans l'île de Procida (Italy), EPPO Publication, European and Mediterranean Plant Production Organization, Paris.

DE MURTAS, ENKERLIN-S., D., CIRIO, U. (in press), Dispersal of Ceratitis capitata (Wiedemann) on Procida island, I. Distribution of sterilized Mediterranean fruit fly on Procida, its relation to doses and feeding, EPPO Publication, European and Mediterranean Plant Production Organization, Paris.

HOOPER, G.H.S. (1970) "Sterilization of the Mediterranean fruit fly: a review of laboratory data", these Proceedings.

HOOPER, G.H.S., NADEL, D.J. (1970) "The pre-release phase of the 1969 Mediterranean fruit fly, suppression experiment in Italy", these Proceedings.

KNIPLING, E.F. (1955) Possibilities of insect control or eradication through the use of sexually sterile males, J. econ. Ent. 48 (4), pp.459-462.

KNIPLING, E.F. (1960) The eradication of the screwworm fly, Sci. Amer. 203 (4), pp.54-61.

NADEL, D.J., GUERRIERI, G. (1969) "Experiments on Mediterranean fruit fly control with the sterile-male technique", Sterile-male Technique for Eradication or Control of Harmful Insects (Proc. Panel Vienna, 1968) IAEA, Vienna, pp.97-105.

NADEL, D.J., MONRO, J., PELEG, B.A., FIGDOR, H.C.F. (1967) A method of releasing sterile Mediterranean fruit fly adults from aircraft, J. econ. Ent. 60 (4), pp.899-902.

RHODE, R.H. (1970) "Application of the sterile male technique in Mediterranean fruit fly suppression: a follow-up experiment in Nicaragua", these Proceedings.

STEINER, E.F., MITCHELL, W.C., BAUMHOVER, A.H. (1952) Progress of fruit fly control by irradiation sterilization in Hawaii and the Marianas Island, Int. J. appl. Radiat. Isotopes 13, pp.427-434.

STEINER, L.F., CHRISTENSON, L.D. (1956a) Potential usefulness of the sterile fly release method in fruit fly eradication programmes. Proc. Hawaiian Acad. Sci. 1955-56.

STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D. (1956b) Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58 (3), pp.519-522.

SOME OBSERVATIONS ON DECREASED VITALITY OF IRRADIATED MEDITERRANEAN FRUIT FLY

A. HAISCH
Bayerische Landesanstalt für Bodenkultur,
Pflanzenbau und Pflanzenschutz,
Munich, Federal Republic of Germany

Abstract

SOME OBSERVATIONS ON DECREASED VITALITY OF IRRADIATED MEDITERRANEAN FRUIT FLY.
It is shown that the irradiation of Ceratitis capitata (Wiedemann) must affect the functions of the digestive apparatus and of the male genital organs. On the whole the competitiveness of irradiated males compared with unirradiated ones was clearly reduced.

The loss of vigour after sterilization is an important matter in the success of the autocidal method. The effects of irradiation dose on some life functions manifesting this vigour have been investigated in Ceratitis capitata (Wiedemann).

1. INGESTION OF ^{32}P-LABELLED MOLASSES

^{32}P-labelled molasses (1 μCi/g) were offered to flies at three defined stages of their life after they had been irradiated, in their late pupal stage, with different doses from a caesium-137 source (71 R/min). The activity of every fly in each experimental unit of 40 individuals was measured immediately after the feeding period.

It is seen from Table I that flies which had been irradiated with 2, 4, 6, 8 or 10 kR showed higher counts than untreated flies. The difference was more marked in those which were fed ^{32}P during the first 3 days than the flies offered ^{32}P during the periods from the 6th to the 9th and from the 12th to the 15th day after emerging.

It is possible that the irradiated flies excrete less than the non-irradiated flies, perhaps because of damaged gut cells. But histological alterations of the mid-gut could not be found.

2. FREQUENCY OF SPERM TRANSFER BY IRRADIATED MALES

Males were irradiated with 2, 4, 6, 8, 10 kR gamma-rays of a ^{137}Cs source (71 R/min) at the late pupal stage. As adults they were caged for 24 hours with virgin females, which were replaced daily by new ones. The sperm content of the spermathecae of these females was examined, giving information on the sexual activity of the males. The sperm content of these was designated normal, small or nil.

No statistically significant (5% level of error probability) differences of sperm transfer between the males irradiated with the dosages of 2 to

TABLE L   RADIOACTIVITY (100 COUNTS PER MINUTE) OF IRRADIATED
FLIES AFTER THEY WERE FED ^{32}P-LABELLED MOLASSES DURING
DIFFERENT STAGES OF THEIR LIFE

Feeding period (days after emergence)	Irradiation dose (kR) [a]					
	0	2	4	6	8	10
1 - 3	41.1 gh	50.9 cde	65.0 a	52.8 cd	59.8 b	45.0 fg
6 - 9	36.2 h	54.3 c	46.7 ef	59.3 b	51.5 cde	48.6 def
12 - 19	40.0 h	41.1 gh	50.3 cde	51.9 cde	49.0 def	66.4 a
Average:	39.13	48.79	53.98 A	54.68 A	53.45 A	53.32 A

[a]   The differences between values characterized by the same letter are not significant (P = 0.01).
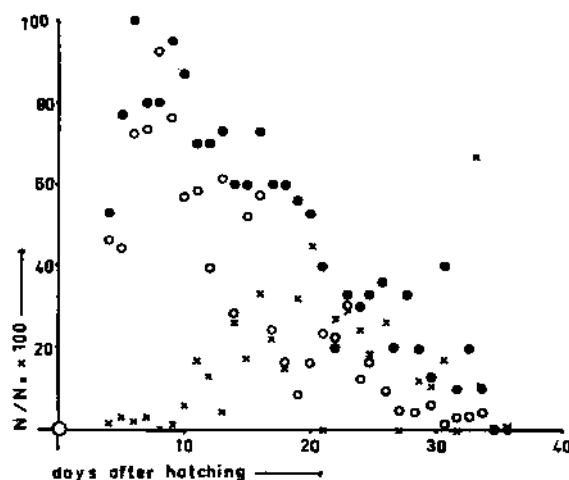
FIG. 1.   Relative frequencies of sperm transfer by males of C. capitata (Wiedemann) in relation to age and irradiation.
N   = number of sperm transfers
N_0 = number of tested flies
●   control males (normal sperm quantity)
O   average (2 - 10 kR) of irradiated males (normal sperm quantity)
×   average (0 - 10 kR) of control and unirradiated males (small sperm quantity)

10 kR could be observed. Therefore, the results of the 2 to 10 kR experi-
mental units are presented (Fig. 1) as an average (open circles). However,
the control males (full circles) copulated significantly (1% level of error
probability) more frequently than the average of the irradiated ones. The
tests were conducted over a period of 36 days. A recovery of the males
was not observed. The results indicate that irradiated males do not
transfer a smaller sperm quantity (×) than control males.

3. OBSERVATIONS ON THE COMPETITIVENESS

The insects were irradiated at the late pupal stage (71 R/min, ^{137}Cs). Then males were caged with normal females. Also, non-irradiated pairs were caged together with either irradiated pairs, irradiated males or irradiated females. In each combination the initial ratio of irradiated to non-irradited males or females was 1:1. The hatching rate of these mingled populations was considered to be a measure of the competitiveness of irradiated males. An undiminished competitiveness of the irradiated males would result in 50% egg fertility according to the general formula

$$f = \frac{q + enp}{en + 1} \tag{1}$$

where f = hatching rate (%) of eggs of the mingled population
    q = hatching rate (%) of eggs of an untreated population
    p = hatching rate (%) of eggs of a population of irradiated and
      non-irradiated females

    $n = \dfrac{\text{irradiated males}}{\text{non-irradiated males}}$

    e = competitiveness of irradiated males.

The formula is based on monogamy of the species. The degree of competitiveness reduction is indicated by the factor e which is 1.0 in the case of full competitiveness and 0.0 in the case of its complete lack.
The standard error of e or its confidence interval was calculated by partial differentiation of

$$e = \frac{q - f}{n (f - p)} \tag{2}$$

with respect to q, f and p:

$$de = \frac{1}{n (f - p)^2} [\partial q (f - p) + \partial f (p - q) + \partial p (q - f)]$$

∂q, ∂f and ∂p are the standard errors of q, f and p multiplied by the relevant t-values (5% level of error probability) so that de equals the confidence interval of e (see Table II).
The crossing of irradiated males and normal females showed hatching results similar to that of Féron (1966)[1] (Table II).
The fertility of populations with females ($i♀ \times n♂♀$) treated with doses of 6 or 8 kR did not differ from the control units possibly because the egg production of these irradiated females was very small.[2] For the same

---

[1] FÉRON, M., Ann. Épiphyties 17 (1966) pp. 229-39.

[2] i = irradiated; n = normal

TABLE II.　EGG HATCHING (%) AFTER IRRADIATION OF THE MALE
PARENTAL PUPAE (AVERAGE OF 2 EXPERIMENTS;　ONE OF THEM
5 TIMES, THE SECOND ONE 4 TIMES REPLICATED;　5 BATCHES OF
20 EGGS PER REPLICATION)

Irradiation dose (kR):	4	6	8	Control
Egg hatching (%)	8.4	3.0	1.3	81.0
Confidence interval* $(t_{0.05} \cdot s_{\bar{x}})$	1.8	0.8	0.7	2.8
Egg hatching (%) corrected to 92.0% control hatching	9.4	3.3	1.6	92.0
Confidence interval* $(t_{0.05} \cdot s_{\bar{x}})$ corrected to 92.0% control hatching	2.0	0.8	0.7	3.0

* $t_{0.05}$ = t-value of 5% level of error probability;　$s_{\bar{x}}$ = standard deviation of a mean.

TABLE III.　RELATIVE FERTILITY (%) OF EGGS AFTER IRRADIATION
IN THE PUPAL STAGE (AVERAGE OF 3 EXPERIMENTS EACH ONCE
REPLICATED;　5 BATCHES OF 20 EGGS PER REPLICATION)

Population		Doses (kR) [a]			
Irradiated	Normal	0	4	6	8
♂	♂♀	90 a	68 de	57 f	72 cd
♂♀	♂♀	92 a	75 bc	60 ef	70 cd
♀	♂♀	93 a	84 b	92 a	91 a
	Average:	92.5	75.7	69.7	77.7

[a] The difference between values characterized by the same letter are statistically not
significant (P = 0.01).

reason, using these doses, fertility rates of populations of normal pairs
kept with only irradiated males (i♂ × n♂♀) or irradiated males and
females (i♂♀ × n♂♀) did not show any significant difference.　The egg
fertility of the two populations was lowest after the irradiation with a dose
of 6 kR.　In applying a 4-kR irradiation dose the higher residual fertility
of the males can be recognized.　These points are shown in Table III.

In the first experiment (first two lines of Table II) the average hatching
rate of the control was 81%, and in the second one (see column 3 of Table III)
it was 92%.　The results (see the third and fourth lines of Table II) were
thus corrected to 92% hatching rate in order to obtain comparable values
for the calculation of e.　The following values of e were then calculated
using the f-values of the (i♂ × n♂♀)-population in formula (2):

Irradiation dose (kR):	4	6	8
Competitiveness, e	0.4	0.6	0.3
Confidence interval, de	< 0.01	< 0.01	< 0.01

The competitiveness of males irradiated with 6 kR is significantly superior to that of males irradiated with 8 kR. It seems that the 6 kR-dose is optimum for sterilization as it apparently produces a minimum of somatic damage (see e) but a maximum of dominant lethals (see f). The low value of e at the 4-kR dose cannot be explained. Perhaps the number of experiments carried out was too small, causing the value to partly reflect secondary influences of environment or development. Thus, the size of each of the three e-values may not be important but the results demonstrate an essential reduction of male competitiveness after irradiation. Still, it should be mentioned that the error in using formula (2) for a polygamous species did not essentially affect the results because the eggs were collected at the beginning of the oviposition period when a second mating perhaps by a fully fertile male was at least not the rule within the experimental populations.

RELEASING SEXUALLY-STERILIZED MEDITERRANEAN FRUIT FLIES FOR CONTROLLING THE INSECT IN NORTH AFRICA

Possibilities of use and problems involved

E.J. HARRIS *
United States of America Agency
for International Development,
Mission to Tunisia

Abstract

RELEASING SEXUALLY-STERILIZED MEDITERRANEAN FRUIT FLIES FOR CONTROLLING THE INSECT IN NORTH AFRICA: POSSIBILITIES OF USE AND PROBLEMS INVOLVED.

The paper discusses the preparations, plans and possible approaches in the work aimed at control of Ceratitis capitata (Wiedemann) in Tunisia and Morocco.

INTRODUCTION

The United States Agency for International Development (USAID) through the United States Department of Agriculture (USDA)[1] is providing assistance to the Governments of Tunisia and Morocco to plan and execute a program for controlling the Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), by using the sterile-fly release method. The first phase of this program will be a feasibility study of the method itself. The second phase will be a full-scale area control program aimed at eradication or reducing medfly populations to the lowest possible levels. A successful outcome will require the establishment of quarantine programs within the participating countries and in co-operation with the Government of Algeria to maintain the gains achieved against this insect. Later, the facilities developed and the personnel employed may be used to control other insects in these participating countries. Where appropriate, conventional control methods (insecticides, poison baits, biological control with parasites and predators, or the use of cultural control practices) will be integrated with the sterile-fly release method to achieve the desired objectives.

The program agreements stipulate that USAID will provide a gamma radiation unit for sterilization and certain other limited supplies and equipment for mass rearing medfly through all stages. U.S. entomologists with experience in using the sterile-fly technique will assist in training other personnel and in organizing, planning, and carrying out the project. The participating countries will provide laboratory facilities, vehicles and personnel to learn and carry out the established procedures. These countries will also take the initiative in carrying out the second phase of the program, and cooperate with other countries wishing to learn the sterile-fly technique of insect control.

---

* Address: c/o American Embassy, Tunis, Tunisia
[1] Technical assistance is provided by the USDA through a Participating Agency Service Agreement.

STATUS AND PLANS

The Government of Tunisia has already built and equipped a new laboratory for mass rearing the medfly. Rearing supplies and equipment, and a cobalt-60 radiation unit has been received and installed. Entomologists and technicians of the National Institute of Agronomic Research in Tunis have started mass rearing and subsequent sterilization and are now ready to begin large-area sterile-fly releases. This is the first preliminary feasibility study of the sterile-fly release method mentioned earlier. These first studies should also include work on comparative mating effectiveness and dispersal of sterilized and wild (feral) flies. In addition, a comparison must be made of the Tunisian and the Vienna fly strains of Mediteranean fruit flies so that the better strain can be used for these area control operations.

Information on the different hosts infested at different seasons of the year in Morocco is limited as is the population dynamics of this insect in Citrus spp. and Argania spinosa. So a study of one year's duration will be made to get this information before the preliminary releases for the evaluation of the sterile-fly technique in Morocco begin.

During this interim period, rearing facilities can be built in Morocco and personnel sent for training in Tunisia. The rearing facilities in Morocco will be at Marrakech. This is highly advantageous, since the place is near the regional agricultural station where citrus is grown, and within driving distance of the Argania spinosa forests. Of the citrus fruits, the sour orange is important because it can provide fruit during the winter in which the fly can breed. The extent to which the Argania fruit contributes to the medfly problem in citrus and other hosts of economic importance should be determined.

Possibilities for using the sterile-fly release technique to control the medfly in Tunisia and Morocco have been assessed and found to be favourable. Although the land area involved is large, the island-like ecological conditions, largely due to large expanses of desert and high mountain ranges, provide the necessary isolation. Natural conditions make this control method even more promising as there are seasonal changes in the climate varying from relative favourability to adversity for fly populations. The test site selected in Tunisia is in the vicinity of Cap Zebib and Ras-El Djebel, consisting of 1500 ha², with over 50% of the area planted in fruit crops. Relative numbers of flies in the area are being estimated now by means of traps baited with male lure. We plan to begin mass releases in January 1970. In Morocco final test-site selections will be based on the preliminary survey of approximately one year's duration.

APPROACHES TO MEDFLY CONTROL THAT APPEAR PROMISING

Research findings from investigations in Costa Rica, Hawaii and in other parts of the world have shown that in general the medfly is affected adversely by irradiation, marking with dyes, and release of adults from airplanes prepackaged in bags or similar containers. We should therefore be sure to use the most suitable radiation dose and timing, and should reduce the handling of the pupae as much as possible after irradiation with

minimal use of dye markers and employment of release methods which give us the best possible adult to compete with the natural populations. Unfortunately, the most practical release method may not give us the most competitive fly.

Although aerial releases enhance dispersal, the methods in use have shown that the handling procedure can cause 50% or more mortality of adults in each container (Harris et al. (1968)). Also, we do not know how much of the effectiveness of the survivors was impaired. After initial primary eradication of melon flies on Rota, Mariana Islands, pupae have been released from well-distributed cages on the ground to eradicate successfully several secondary re-infestations of this species (Steiner et al. (1965, 1970)). If dispersal by this method can be shown to be satisfactory in Africa, releases of pupae rather than aerial releases of adults may well be the method that will give the most feasible control. We believe that, to get satisfactory distribution of flies over a large area, ground releases may not be practical. However, in case aerial release of adults is only partially effective we may have to use selected ground release sites for pupae.

The following control procedures should also be considered:

(1) Dispose or bury cull fruit in orchards and farmyards (commodity sanitation).

(2) When practical, destroy non-economic hosts mechanically or with herbicides to interrupt the sequence of host attack during unfavourable periods.

(3) Monitor fly populations in the most favourable fly population areas (hot spots) and apply insecticides or poison baits during that period when feral populations are at their lowest levels. This procedure may appear to be the reverse of the usual practice, but may result in drastic reductions of future high population levels.

(4) Evaluate the possibility of reducing medly populations in selected areas where fly production is high in the major host Argenia spinosa, which produces fruit throughout the year, by herbicide treatment to cause fruit droppage.

(5) Evaluate the possibility of releasing the Hawaiian opiine parasites, Opius oophilus, O. longicaudatus, and O. vandenboschi, to control the medfly in Argania spinosa.

CONCLUSIONS

In North Africa, the seasonal changes in climate, host, and fly abundance create the possibility of replication of test sites in number and size. This is an advantage not possible in some other areas where previous studies of the method have been made. Comparisons are possible involving the use of sterile releases, insecticides or poison baits, or combinations

of the two control methods. Because of cost factors and logistics problems, we may not be able to pursue all of the options open to us. Most of all, we need to maintain flexibility to make changes when necessary to use the latest information gained from research in Hawaii, Austria, Tunisia, or elsewhere and experience gained in Tunisia.

REFERENCES

HARRIS, E.J., MITCHELL, W.C., BAUMHOVER, A.H., STEINER, L.F. (1968). Mutilation and survival of sterile oriental fruit flies and melon flies emerging in drop boxes, J. econ. Ent. 61, pp.493-96.

STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D. (1965). Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58, pp.519-22.

STEINER, L.F., HART, W.G., HARRIS, E.J., CUNNINGHAM, R.T., OHINATA, K., KAMAKAHI, D.C. (1970). Eradication of the oriental fruit fly from the Mariana Islands by male annihilation and sterile insect release, J. econ. Ent. 63, pp.131-35.

MEDITERRANEAN FRUIT FLY: SHORT CONTRIBUTIONS

Summaries of work done
at various institutions

SOME EFFECTS OF GAMMA RADIATION ON THE SEXUAL
VIGOUR OF Ceratitis capitata (WIEDEMANN)
K.P. Katiyar, E. Ramírez
Inter-American Institute of Agricultural Sciences of the OAS,
Turrialba, Costa Rica

Competition between irradiated and normal sperm in fertilization

Sequential matings of the medfly female with normal and 10 kR-irradiated
males indicated that normal males were somewhat more aggressive in mating
than irradiated males. More females mated a second time (41.3%) with
normal males when the initial mating was with an irradiated male. Fewer
(26.7%) females mated a second time with irradiated males when the original
mating was with a normal male.

Fertility of the females mated twice, once with normal and once with
irradiated male, showed that mixing of sperm from two matings occurred
inside the female reproductive system. Second mating did not completely
nullify the influence of previous insemination. Second mating with normal
males was more effective in altering female fertility than second mating
with sterile males. The fertility of females alternately mated to both types
of males increased 82.1% when the last mating was with a normal male and
decreased 51.6% when the last mating was with an irradiated male. Perhaps
sperm from irradiated males is somewhat less competitive than from normal
males, especially when the last mating is with normal males.

Sterilization levels and male sexual vigour

Sterilization of the medfly males (at pupal stage, 24 hours before adult
emergence) with 8 kR and 10 kR seems to reduce slightly the mating vigour
of the treated males. The sexual vigour of males receiving 6 kR is not
adversely affected. Five hundred virgin males (125 of each type: 6 kR,
8 kR, 10 kR and normal) were simultaneously released with 250 virgin
normal females in caged coffee trees. Of the total observed matings (100%),
22.5% and 23.0% were by males irradiated with 8-kR and 10-kR doses
respectively, compared to 27.7% and 26.6% by 6-kR-irradiated and untreated
males respectively.

In another test, the mating competitiveness of medfly males irradiated
with various sterilization doses (5 kR, 7 kR, 9 kR and 11 kR) was measured
in terms of reduction in fertility of normal females when treated males
were caged with normal males and normal females at a ratio of 5 : 1 : 1.
Higher sterilization dose did not show reduction in mating competitiveness of
irradiated males. The lowest sterilization dose (5 kR) and the highest dose
(11 kR) gave 30.2% and 32.2% egg viability of the normal flies respectively.

Stage of sterilization and male sexual vigour

Mating vigour (insemination efficiency) of 10-kR-irradiated males was
affected by the pupal stage at which irradiation is applied. The closer to
adult emergence that the pupae are irradiated, the higher is the insemination
efficiency of the treated males. During 4 weeks of adult life, males
irradiated 72 hours before adult emergence showed 75% reduction in

insemination efficiency compared to the males irradiated 24 hours before adult emergence. Further experiments also showed increase in mating efficiency (in terms of numbers of inseminations) by males irradiated during the adult stage (24 hours or 48 hours after emergence) compared to males irradiated as pupae (24 hours before emergence). However, when mating competitiveness was measured in terms of reduction of normal female fertility, there was no difference in sexual vigour of the males irradiated either as pupae or as adults. Overflooding normal populations with sterile insects at a ratio of 40 : 1 gave 1.6% egg-hatch when males were irradiated as pupae (24 hours before emergence) compared to 1.7% egg-hatch when males were irradiated 24 hours after emergence.

Population suppression by sterile-male releases in caged coffee trees

Suppression of the reproductive potential in medfly by gamma-irradiated males through weekly releases of treated (7 kR applied at pupal stage, 24 hours before emergence) and untreated wild flies (both sexes) in caged fruiting coffee trees was tested at three overflooding ratios (20 : 1, 40 : 1 and 80 : 1). The first fly release consisted of 20 normal wild flies (10 males and 10 females) per ratio in each cage. In subsequent releases, the number of adults was reduced to half.

Examination of the mature coffee berries for egg-hatch over 4 weeks indicated that irradiated males were not equally competitive in mating with normal wild flies. Release of irradiated flies with normal wild flies at a ratio of 80 : 1 was most effective in suppressing the reproductive potential of normal flies. Such a mixed fly population laid over a 4-week period an average of 15.4% viable eggs (the egg-hatch in check was 99.0%). Releases of sterile flies with normal flies at ratios of 20 : 1 and 40 : 1 lowered the fertility of normal flies to 23.2% and 26.6% respectively. This test is being repeated.

INVESTIGATIONS CARRIED OUT IN SUPPORT OF THE MEDITERRANEAN FRUIT FLY STERILE FLY RELEASE EXPERIMENT IN NICARAGUA

R.H. Rhode
IAEA, San José, Costa Rica

Laboratory

An increase in fly stock longevity which resulted in recovery of 50% more eggs/female was obtained by holding the flies in cages measuring 2.40 m × 0.60 m × 0.35 m. The roof, floor and three sides are covered with 18-mesh plastic screen. The remaining side is covered with white dacron cloth as an oviposition surface. This cage replaces the Modified Hawaii type (1.2 m × 0.6 × 0.3 m) equipped with oviposition cloth on one side.

A carrageenan (Gelcarin HWG, Marine Colloids Inc.) at 1% in water was found to be as effective as agar in providing moisture for the stock flies. Its cost is about half that of agar.

Preliminary results using fresh residual brewer's yeast ordinarily discarded by a local brewery, in place of torula yeast, in the larval media gave promising results. However, because of its liquid state, new handling methods must be developed before its use on a large scale becomes practical. Studies are underway to determine whether crude sugar-cane molasses can be used effectively in the larval medium. This material is plentiful and much cheaper than the refined sugar now employed.

Field

Results of large field cage tests comparing our laboratory strain with that of Vienna revealed no differences in sterile-male mating aggressiveness when both types treated with 8000 rad were exposed to untreated wild females.

In each of three cages, 500 sterile laboratory males and 500 sterile Vienna males were placed with 500 untreated wild males. Each day for 5 days 150 females of both the laboratory and Vienna strains were introduced along with 150 untreated wild females. Samples of copulating pairs were taken during a 2-hour period each day. The experiment was repeated 15 days later.

Combined data of the two tests indicate a mean total of 89.5 matings for the sterile Vienna male-wild female combination. An identical number of matings were also obtained for the sterile laboratory male-wild female cross. The number of these matings compared favourably with the normal wild male-wild female total mean of 111.5 matings.

Over a 4-month period 297 000 582 laboratory and 304 337 628 Vienna strains of sterilized Medflies were released in Nicaragua. Mean emergence rates were 88.3% and 87.8% respectively for the laboratory and Vienna strains. Of the 148 500 291 effective laboratory males released, 313 380 or 0.211% were recovered compared with 394 469 or 0.259% of the 152 168 814 effective Vienna males. Assuming an equal lure response, no significant differences apparently exist between the two strains in their ability to survive under the environmental conditions of the experimental area.

In aerial release experiments carried out near San José with USAF co-operation, several fly dispersing techniques were tested. One treatment consisted of wood-excelsior-filled bags being slit along two or three sides as they left the ejection tube. An X-shaped cardboard partition was placed in other bags. In a third method, lightweight cardboard tubes 1 m long and $3\frac{1}{2}$ cm in diameter were joined together in groups of 14 to form a cluster. The flies from these tubes were ejected directly into the airstream with no protective covering. Approximately 195 000 effective males were used for each treatment with a resting surface of at least 1 cm^2 per fly being provided in each type of container. The wood excelsior and X-insert bags were ejected simultaneously using two release chutes. Unfortunately, the knife blades on the X-insert chute were set too deep causing the majority of these bags to be torn completely apart. As no radio communication with the plane was maintained the condition could not be corrected.

The test site – a coffee farm – is roughly in the shape of an hour-glass and varies from 150 to 300 m in width. Releases were made using a C-47 aircraft flying less than 30 m above ground level at an indicated air speed of around 130 knots. Flies were dropped along an 800-m-long line bisecting the test plot. Wind was nil or light during all tests. Trap lines were set up

TABLE I. PERCENTAGES OF FLIES TRAPPED BASED ON MEAN FLY CATCHES OF TRAPS LOCATED AT 50 m, 100 m AND 150 m NORTH AND SOUTH OF THE RELEASE LINE

Trap distance	Percentage trapped		
	Excelsior bags	X-insert bags	Tubes
50 m (20 traps)	72.4	67.6	62.5
100 m (18 traps)	17.6	18.6	22.6
150 m (8 traps)	9.9	13.7	14.8

TABLE II. FLIES PER TRAP DAY CAPTURED AT 50 m, 100 m AND 150 m NORTH AND SOUTH OF THE RELEASE LINE AT INDICATED TIME AFTER DROP

Trap distance	Flies per trap day at post-drop interval (days) of:							
	2 d	3 d	4-6 d	7-8 d	9 d	10-12 d	13-14 d	15-16 d
50 m	51.1	55.8	41.3	13.0	12.3	1.5	1.2	1.2
100 m	8.7	9.8	12.1	5.8	4.7	0.8	0.7	0.5
150 m	12.1	4.0	7.8	2.5	3.1	0.8	0.5	0.4

perpendicular to the flight path at 50-m intervals. In each line traps were placed 50 m apart. A total of 71 traps were employed. In about two-thirds of the drop area the coffee is grown under shade trees; no cover shade is present in the remaining one-third of the plot. Coffee was maturing at the time of the test and honey-dew-producing insects were in evidence.

Eight trap examinations were conducted throughout a 16-day post-drop period. Traps were placed in operation 24 hours after the drop. Percentages of flies recovered of those released in each treatment were: slit bags, 5.1; X-insert bags, 4.3; and cardboard tubes, 3.3.

Although the experiment was not designed to study fly movement specifically, some pertinent data were obtained from the test. Table I, based on trap catches of the combined treatments, show that under these test conditions the majority of flies captured were taken near their point of release. The trap catches were effected in the shade-grown coffee area.

Table II presents catches of the combined treatment obtained at the various distances on a fly per trap day basis at various post-drop periods.

The similar rates of decline in fly captures at the various distances do not signify a regular shifting movement of flies en masse outward from the release line over the trapping period.

At the same experimental site two other tests were conducted to compare the effectiveness of flies released by air from the C-47 aircraft with bagged flies released on the ground. The first test was originally designed to compare two aerial dispensing techniques using laboratory flies and at the same time to compare the survivability of the Vienna and laboratory strains of flies released on the ground. Two of the fluorescent dyes used, however,

were indistinguishable in small amounts, making positive identification impossible. Therefore aerial-released laboratory flies and ground-released Vienna flies only could be compared. These two fly strains are similar, as release data gathered in Nicaragua indicate. Of the approximately 457 000 effective laboratory males released by air, 0.85% were recovered compared with 1.62% of the 471 000 Vienna males released on the ground. As approximately equal numbers of each category were released, recoveries from aerial-released flies were about 47.5% less than from ground-released flies.

In the second test approximately 350 000 effective males were released from the C-47 using the slit bag method. An equal number of males were released from bags distributed on the ground along the length of the flight line. A total of 6872 aerial-released flies were recovered compared with 17 149 ground-released flies captured, indicating that recaptures of aerial-released flies in this test were about 60% less than those of ground-released flies. Sterile flies were used in all release experiments.

Nine pupal and adult aerial releases were made in the 48-km^2 test area in Nicaragua. About 12 000 000 pupae and an equal number of adults were released twice a week from 28 May to 26 June 1969. The results of these drops are somewhat difficult to evaluate. In spite of our efforts to ensure that the bags of flies were torn completely open when expelled, a number of traps in the release area captured from 100 up to 3000 marked flies per week. This indicates that bags with flies still remaining in them fell close to these traps. Such a high concentration of flies is not as likely to occur in one spot as a result of the pupae releases. Their release rate was calibrated at about 3600 pupae/sec during which time the plane covered a distance of about 65 m. Separate arrays were made of the flies caught in each trap, which consisted of marked flies (released as adults) and unmarked flies (released as pupae). Traps with zero catches of either category were eliminated. The medians of each array were established and compared. In this manner the number of unmarked flies captured was around one-third that of the marked flies recovered at a trap density of 3.2 traps/km^2.

NOTE ON WORK AT ISPRA

R. Cavalloro
EURATOM Joint Nuclear Research Center,
Ispra, Italy

Ceratitis capitata (Wiedemann) lends itself easily to mass rearing in the laboratory. Practical improvements of the rearing techniques have been obtained, especially with respect to the diet. Several medfly strains from various geographical origins around the Mediterranean basin have all responded well to diets based on alfalfa meal and ground cornstalks.

Considering the yield of pupae, their unit weight, the percent of emergence, the vitality of the adults and other parameters, it seems most advantageous to place 30 000 eggs per kg of pabulum. The larvae mature one day earlier on the cornstalks than on the alfalfa diets.

A new type of cage for adults has been built and the automation of certain operations has been considered, especially with regard to the collection of the eggs and their transport to the larval pabulum.

ACTIVITES DE RECHERCHES DU LABORATOIRE D' ENTOMOLOGIE
DE L'INSTITUT NATIONAL DE LA RECHERCHE
AGRONOMIQUE DE TUNISIE

M. Cheikh
Institut National de la Recherche Agronomique de Tunisie,
Ariana, Tunisie

Introduction

La mouche méditerranéenne des fruits occupe une place très importante dans la recherche agronomique tunisienne.

Depuis bientôt 12 ans, le laboratoire d'entomologie a entrepris des travaux en vue de l'application d'une méthode de lutte autocide contre Ceratitis capitata.

L'INRAT avait déjà bénéficié d'un contrat de recherche de l'AIEA (1963-1965). En 1968, l'USAID et l'USDA ont mis à la disposition de l'Institut les moyens nécessaires à l'exécution d'un programme de lutte par lâchers de mâles stériles dans la zone de Ras-Djebel dans le nord du pays. La zone choisie présente des conditions d'isolement et d'études très intéressantes. Elle couvre 3000 ha environ de cultures fruitières diverses.

Elevage industriel

Un nouveau laboratoire a été récemment construit. Il couvre 500 m², et comprend 17 pièces, réparties entre un rez-de-chaussée et un étage et réservées exclusivement à la production de masse de l'insecte qui doit atteindre 3 millions de mouches/jour. Ce bâtiment est doté d'un irradiateur au ^{60}Co de 1500 Ci offert par l'AID.

Un nouveau type de cage est mis en service. Il est en tulle moustiquaire avec cadre suspenseur en bois supportant un pondoir et cuvette à fond grillagé sur lequel sont placés 400 g de sucre en morceaux.

Ce type de cage offre les avantages suivants:
- souple et lavable
- économique pour le prix de la toile et pour sa couture (faite au laboratoire par la machine à coudre achetée récemment pour la fermeture des sachets en papier Kraft servant au lâchers)
- la cage reste fermée pendant toute la vie des insectes qui disposent de l'alimentation à demeure. La récolte des œufs se fait par jet d'eau qui s'écoule à l'extérieur par des tuyaux d'évacuation.

Nous pensons dans l'avenir faciliter la collecte des œufs en adaptant aux pondoirs un système d'irrigation; les tuyaux d'évacuation de deux éléments de trois cages déboucheraient dans une conduite unique au bout de laquelle se ferait la collecte des œufs.

En ce qui concerne l'élevage des larves nous utilisons la formule SYGS (sucre, levure, gelgard, son) mise au point par la Station de Hawaii (Steiner 1968). Le milieu d'élevage est mis dans des cuvettes de fabrication américaine de 40 × 75 cm et 35 mm de profondeur, empilables sur chariot, donc faciles à déplacer. Ces cuvettes présentent des échancrures latérales qui permettent aux larves d'accomplir leurs sauts.

Néanmoins, certaines conditions sont à éclaircir dans l'avenir, à savoir:
- l'influence de l'humidité sur les différents stades larvaires
- l'épaisseur du milieu
- le refroidissement du milieu qui s'échauffe, pendant les derniers jours précédant le saut; cet échauffement dépend de l'épaisseur du milieu et de la largeur de la cuvette
- l'influence du rythme nycthéméral et l'intensité d'éclairement sur le saut des larves
- l'influence de l'hygrométrie relative de l'air ambiant; cette hygrométrie peut être un facteur important dans le déclenchement du saut et de la nymphose en dehors du milieu d'élevage larvaire.

Nymphose: La nymphose se fait dans le sable; la séparation des pupes se fait par tamisage. Nous envisageons la construction d'un tamis mécanique à débit continu. Il serait bon d'entreprendre des recherches sur l'influence de la température et de l'humidité sur le déclenchement de la nymphose.

Irradiation: Des essais sont en cours pour tester la stérilité des insectes irradiés et le contrôle des femelles stérilisées au point de vue comportement de ponte.

Histologie de l'adulte

- Etude des effets histopathologiques des irradiations
- Interaction des irradiations et des températures basses (températures entre 11° et 20°) sur la stérilité.

Nous pensons développer ce programme en vue d'un éventuel stockage des pupes irradiées ou non.

Etude de la dispersion

Nous avons commencé les lâchers qui ont été interrompus à la suite du déménagement dans le nouveau laboratoire. Nous envisageons la poursuite des lâchers d'insectes stériles pour apprécier leur pouvoir de dispersion.

Aptitude à l'envol

Nous utilisons une cage très haute baptisée cage «gratte-ciel» de 2, 80 m de hauteur, 90 cm de longueur et 55 cm de largeur (Soria - Yana 1969) à deux compartiments juxtaposés et divisé chacun en cinq étages. Nous testons l'influence de l'âge, du sexe et du facteur alimentation sur l'aptitude à l'envol des souches différentes et irradiées aux différentes doses.

Etude de la longévité des mouches d'élevage dans les conditions extérieures

Nous maintenons des lots de mouches à l'extérieur du laboratoire et notons la mortalité des insectes fertiles et stérilisés aux différentes doses.

Piégeage

Une équipe intensifie le piégeage dans la zone pilote de Ras-Djebel.
Les différentes stations sont visités et les captures dénombrées une fois

par semaine. Depuis août 1968, on a commencé le piégeage par pièges
Steiner à 200 m d'intervalle.

Programme d'avenir: Il nous reste, suivant nos moyens en personnel
qualifié, à tester la compétitivité de nos insectes irradiés, à mettre au
point une technique de lâchers aérien en collaboration avec la Société
Nationale de la Protection des Végétaux, à effectuer la sélection de popula-
tions résistantes aux insecticides et à pouvoir poursuivre et développer le
programme actuel de recherches.

WORK AT THE INSTITUTO NACIONAL DE INVESTIGACIONES AGRONOMICAS, MADRID

L. Mellado, M. Arroyo, A. Jimenez
Instituto Nacional de Investigaciones Agronómicas,
Madrid, Spain

The I.N.I.A. started in 1965 a Program of application of the sterile-
male technique for the control of Ceratitis capitata (Wiedemann). The
research, and field work on the island of Tenerife, are summarized in the
following points.

1.   Laboratory work

1.1. 1965–66. Mass rearing of C. capitata was started. A daily average
of 53 000 pupae was reached. Basic studies on radiation sterilization,
tagging with radioisotopes and rearing techniques were carried out.[1]

1.2. 1967. A new laboratory for mass rearing was set up. Research on
feeding diets and oviposition was carried out. A daily average of
72 000 pupae was obtained.

1.3. 1968. Production of pupae rose to 200 000 per day, due to the new
rearing methods applied. In 1969, during the period January–July, the
daily production of pupae averaged 400 000.

2.   Field work

2.1. Island of Tenerife

2.1.1. In 1966, the island of Tenerife was chosen to start tests on the mass
release of sterilized flies. Within that island, two adjacent and relatively
isolated valleys were selected, one as experimental area and one for control.
There were no regular plantations in either area. No pesticide treatments
were done. Main host plants were: figs, grapes, citrus, apricots and
peaches. A secondary host plant was Oppuntia ficus-indica. Both areas
suffered endemic and heavy attack from the fruit fly.

---

[1] ARROYO, M. et al., Bolet. Patolog. Veg. Entom. Agric. XXVIII (1965) 257-280.

2.1.2. Release of sterile insects was started in May 1966, over an area of about 450 ha. Pupae, irradiated in Madrid, at a dose of 9000 rad, in a ^{137}Cs source, were sent by commercial airline to Tenerife. Different methods of shipment and release were tested. Only ground release was done. During the period May–December, a total of more than 7 million sterile insects was released. No effective control was obtained, the infestation continuing to be very heavy.[2]

2.1.3. In 1967, a total of four million sterile flies was released in the same area. No systematic control of infested fruit was carried out. However, some tests showed a decreased infestation in the release area.[3]

2.1.4. In 1968, 25 million sterile insects were released in the same area. A control of infested fruit was carried out on peaches. In the release area, infested fruit averaged 15% (ranging from 6% to 23%). In the control area (no treatments), the average of infested fruit was 70% (ranging from 39% to 100%).

A summary of the field work on the Spanish mainland is given in the next contribution.

MEDITERRANEAN FRUIT FLY SUPPRESSION EXPERIMENT ON THE SPANISH MAINLAND IN 1969

L. Mellado[*], D.J. Nadel[†], M. Arroyo[*], A. Jimenez[*]

In 1969, the Tenerife program was temporarily abandoned owing to mainly logistic difficulties similar to those encountered elsewhere. A new experimental site was chosen on the Spanish mainland in a semi-isolated area at Alhama de Murcia containing a total of 25 ha of regular plantations of citrus varieties and apricots and peaches. Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), is endemic in the area, and a regular plantation of peaches in which the fly was controlled by insecticide treatments, and eight other untreated areas located one or more kilometres from the release site, were used as controls.

The objectives of the program were, with the minimum of labour available, and without any other treatment, to attempt to suppress medfly in the experimental area through the release of sterile flies. The extent of stinging of host fruit by sterile female flies was also to be assessed.

The Seibersdorf Laboratory of the International Atomic Energy Agency co-operated in the experiment with technical advice and supplying pupae during the first part.

[2] MELLADO, L. et al., Bolet. Patolog. Veg. Entom. Agric. XXIX (1966) 89–117.
[3] ARROYO, M. et al., Bolet. Patolog. Veg. Entom. Agric. XXX (1967–68) 233–249, 251–285.
[*] Instituto Nacional de Investigaciones Agronómicas, Madrid, Spain.
[†] International Atomic Energy Agency, Vienna

All the pupae were irradiated in Madrid with a target dose of 9 krad the day before the first emergence was expected, and were transported by car to Murcia several times weekly in special containers. At the experimental site the irradiated pupae were repackaged in perforated paper bags in which the flies emerged. The flies were subsequently released at pre-determined sites.

Releases of sterile flies were carried out from 17 March to 4 August. A total of 32 million pupae were irradiated for use in the experiment, 26 million from the I.N.I.A. laboratory in Madrid and the remainder from the Joint FAO/IAEA Seibersdorf Laboratory (air-transported once weekly as young pupae).

The experimental results were determined in a number of ways, including the examination of fallen fruit, visual examination of all host-bearing trees, and a close examination of all fruit that appeared to be stung; and by determining the percentage of fruits at harvest damaged by medfly. To ensure a continuing supply of fruit for examination, selected trees were not harvested.

Studies of the dispersal and longevity of marked flies were also carried out using cylinder-type traps with trimedlure as the attractant. When the bags were opened the flies escaped rapidly and the trapping data showed that subsequent longevity and dispersion were satisfactory.

Although the experimental area was semi-isolated, it was shown that, given favourable conditions, gravid females from nearby control areas could migrate into the experimental area.

Before the initial release of sterile flies, it was found that 10% of fallen fruit of already ripe citrus were infested, although there was no infested fruit found on the trees.

In the experimental areas a single apricot was found to be infested from the total harvest of 242 bearing trees. Of the 40 000 kg of peaches inspected at harvest, approximately 8 kg (0.02%) were infested. No sterile stings were found in the harvested citrus and the percentage of such stings in apricots and peaches was small.

In the insecticide-treated peach orchard, neither stings nor infested fruit were found throughout the period of the experiment. In the untreated control areas, wild flies were first trapped on 13 May and the first stung and infested fruits were found at the beginning of July. By the end of July all the fruit found on or under the trees had been attacked.

A full account of the experiment will be published elsewhere.

RECENT RESEARCH ON STERILIZATION OF Ceratitis capitata (WIEDEMANN) AT THE SEIBERSDORF LABORATORY OF THE IAEA

G.H.S. Hooper
International Atomic Energy Agency, Vienna

Recent research has been devoted to accumulating replicated data, suitable for statistical analysis, on (a) the relation between irradiation dosage and sterility, (b) the competitiveness of males at various dosages as measured by ratio tests and (c) the effect of alternately mating females with irradiated and untreated males.

Materials and methods

The materials and methods used were as follows.

(1) All pupae were taken from the mass production unit, and were stored before and after irradiation at 25°C.

(2) Irradiation was achieved with a ^{60}Co Gammacell 220 having a dose rate in the region of 9.2 krad/min with an error of ± 5%.

(3) Pupae were irradiated, and all tests used adults which emerged 24 – 48 hours after irradiation.

(4) In general, tests were replicated 5 times, one replicate being set up per day.

(5) Most work has been devoted to male sterility, evaluated egg hatch of the cross irradiated male X untreated female; test eggs were kept on moist filter paper and hatch assessed after 5 days.

Dosage – sterility studies

In the course of three experiments, dosages from 1 to 13 krad have been used. Egg hatch from 7 to 7.5 krad has been consistently below 5% (corrected for check) and for 9 krad has been between 1 and 2%. Even at 13 krad total sterility of males was not obtained.

At the conclusion of one experiment, all surviving irradiated males, which were 39 days old, were mated with young virgin untreated females. Egg hatch data collected when the males were 49 – 61 days old gave no indication of recovery of fertility at the dosages employed (7.5 – 13 krad).

In a limited test (2 replications) irradiated females, crossed with untreated males, produced no eggs at 3 or 5 krad. The eggs produced at 1 krad were of normal fertility. However, data from other experiments have shown the females irradiated with up to 13 krad could produce some unfertile eggs.

Ratio tests

An attempt was made to measure the combined effect of male sterility and competitiveness, at a number of dosages, on egg hatch. Males irradiated at dosages from 3 to 17 krad were combined with untreated flies in the ratio 150 irradiated males: 30 untreated males: 30 untreated females (i.e. 5:1:1). We interpreted the egg hatch data obtained in the following way: at 3 and 5 krad the males were incompletely sterile but competitive; at 7, 9 and 11 krad the males were at least 95% sterile but were less competitive than untreated males; at 13, 15 and 17 krad, the males were for practical purposes sterile but the competitiveness of these flies was markedly and progressively depressed.

A further experiment was run using a 19:1:1 ratio (475 irradiated males: 25 untreated males: 25 untreated females). There were no great differences in the egg hatch produced by 5, 7, 9 and 11 krad, but statistical analysis may indicate that 7 krad was the optimum dosage.

In a ratio test using flies irradiated at 9 krad, and with approximately 10 000 flies per treatment, a ratio of 49:1 (irradiated males and females: untreated males and females) gave a corrected egg hatch of 8.8% and a ratio of 98:1 gave 5.4%.

In small cage tests with a 1 : 1 ratio there was little difference in corrected egg hatch between 7, 9 and 11 krad (77, 76 and 87% respectively). The hatch was considerably higher than that expected for a 1 : 1 ratio.

Alternate mating test

When 9-krad-irradiated males were mated with untreated females the egg hatch was 2.2%. After 10 days the irradiated males were replaced by untreated males and the egg hatch over the next 17 days was 28.7% – an increase of 25.5%. When untreated males were mated with untreated females the egg hatch was 90.1% and when these males were replaced by irradiated males 10 days later the egg hatch decreased by 23.7% to 76.4%.

RECENT WORK ON PHYSIOLOGY OF THE MEDITERRANEAN FRUIT FLY AT THE SEIBERSDORF LABORATORY OF THE IAEA
P.A. Langley *
International Atomic Energy Agency, Vienna

The rate of fat consumption and water loss by pupae has been determined at 25°C and 75% r.h. Water loss is greatest during the first 3 days of pupal life, after which the rate falls to a low level and rises again the day before adult emergence.

Adult emergence is slightly reduced when pupae are incubated in 30% r.h. at 20°C (82% emergence, compared to 91% and 96% emergence at 75% and 90% r.h.). At 30°C, 78% and 75% emergence is obtained in 30% and 75% r.h., while only 18% emergence is obtained at 90% r.h. owing to fungal growth on pupal shells. More crippled flies are produced in low r.h. than in higher r.h.

The fat content of pupae falls from about 40% of the residual 'non-fatty' dry weight (RDW) to 15% of the RDW throughout development at 25°C and 75% r.h.

The length of pupal life is affected by temperature in a curvilinear fashion. At mean incubation temperatures higher than 25°C the fat contents of emergent flies are reduced. At 30°C the fat reserves approach those of flies which have been starved to death.

The Respiratory Quotient (R.Q.) of the pupa is about 0.7, indicating that fat is the most important energy reserve.

The R.Q. of the recently emerged adult increases from 0.9 to 1.55 during the next 48 hours and is unaffected by allowing the insects to feed on sugar and water. This indicates that carbohydrate is being converted to fat in both the unfed and fed flies.

Measurements of total oxygen consumption during pupal development, together with fat consumption, allow the estimate that 1850 mm^3 of oxygen $\equiv$ 1 mg fat to be made.

An average oxygen consumption of 12 mm^3/h by recently emerged and unfed adults enables an estimate of their survival time to be made at 25°C on the basis of their fat reserves. This is theoretically 48 h if pupae were incubated at 25°C, but falls to 10 h if pupal incubation occurred at 30°C.

* Present address: Tsetse Research Laboratory, Dept. of Veterinary Medicine, University of Bristol, Langford House, Langford, Bristol, United Kingdom.

In practice survival is a little longer, indicating the presence of other reserves and confirming the high R.Q. values noted in adults.

Experiments have shown that irradiation of pupae on the 4th day of development with 10 krad of gamma rays at 25°C permits almost 100% to complete development, but emergence of adults is less than 20%. Irradiation on the 6th day of development allows almost 100% emergence. This 2-day difference in age which permits 100% emergence is reduced to nil if irradiation occurs in nitrogen. In nitrogen 100% emergence occurs in pupae irradiated on the 4th day of development. Irradiation of pupae cooled to 4°C produces the same 2-day interval as in controls.

The effect of nitrogen is even more striking with doses of 30 krad. 100% development occurs in pupae irradiated on day 5 but 100% emergence is only achieved in pupae irradiated on the 9th day. This 4-day interval is reduced to 1 day if pupae are irradiated in nitrogen, but if pupae are cooled to 4°C before irradiation the 4-day interval remains.

Sterility of adults emerging from the pupae in the irradiation experiments was assessed by checking egg hatch from equal numbers of normal virgin females placed with males emerging from irradiated pupae. Egg collections were made 5 times during 3 weeks following emergence. Egg hatch varied from 0 - 2% in experiments involving irradiation at 30 krad. Egg hatch varied from 0 to 7% in experiments using 10 krad. No differences were noted which could be attributed to the effects of nitrogen or chilling.

It is concluded that lethal side effects of gamma irradiation which are oxygen-dependent, are reduced in the presence of nitrogen without affecting the damage to genetic material in the reproductive tissues.

Survival data on adults irradiated as pupae as described, have been collected but are not yet available. Indications are that survival of adults irradiated early in the pupal stage is not as good as those irradiated later.

FRUIT FLIES OTHER THAN MEDITERRANEAN FRUIT FLY: REPORTS

STERILE-INSECT TECHNIQUE
FOR ERADICATION OR CONTROL OF
THE MELON FLY AND ORIENTAL FRUIT FLY*

Review of current status

D.L. CHAMBERS, N.R. SPENCER,† N. TANAKA,
R.T. CUNNINGHAM
Entomology Research Division,
Agricultural Research Service,
United States Department of Agriculture,
Honolulu, Hawaii, United States of America

Abstract

STERILE-INSECT TECHNIQUE FOR ERADICATION OR CONTROL OF THE MELON FLY AND ORIENTAL FRUIT FLY.

A summary is presented of the programs conducted in the Mariana Islands to develop the method of insect control by releasing sterile insects by the Hawaiian Fruit Flies Investigations, Agricultural Research Service. Control and eradication of the melon fly and oriental fruit fly has been achieved, and successful completion of a current program of eradication of the melon fly from Guam will free the Mariana Islands of pest tephritids.

The use of the method of sterile-insect release to eradicate and control the oriental fruit fly, Dacus dorsalis Hendel, and the melon fly, D. cucurbitae Coquillett, was described in a series of publications by L. F. Steiner and associates. These programs were conducted on the island of Guam, Rota, Saipan, Tinian, and Agiguan, in the Southern Marianas. The first attempt, against the oriental fruit fly on Rota (1960-62), was unsuccessful because adequate overflooding could not be achieved (Steiner et al. (1962)). However, the method was used subsequently (1962-63) to eradicate the melon fly from Rota (Steiner et al. (1965a)) at the same time that the oriental fruit fly was eradicated from that island by the method of male annihilation (Steiner et al. (1965b)). The pupae used in the melon-fly releases were produced at the Hawaiian Fruit Flies Investigations Laboratory in Honolulu, irradiated there with 9.5 kR in a cobalt-60 irradiator, and shipped by air for release on Rota. A total of about 257 million flies was distributed from air and on the ground in weekly releases.

The second attempt to eradicate the oriental fruit fly by the sterile-insect release method was begun on Guam in September 1963 (Steiner et al. (1970)); by February 1964, about 16 million flies had been released, and the native population was considered eradicated. However, the following spring and summer, single wild flies were captured on four occasions;

---

therefore additional releases of about 200 000 sterile flies were made
in the vicinity of each recovery site. The following year another small
outbreak was found and it was first controlled with bait sprays and wafers
impregnated with methyl eugenol and then eradicated by making weekly
releases of 9.5 million sterile flies.

On Saipan, Tinian, and Agiguan, releases of sterile oriental fruit
flies were begun in February 1964. On Saipan, the flies were distributed
as pupae which were placed in emergence cages, but aerial distribution
was necessary on Tinian and Agiguan. About 2.5 - 4 million pupae that
had been reared and irradiated at the Honolulu laboratory were released
each week on Saipan for nearly 1 year. During the same period, about
1 million flies were released weekly on Tinian and Agiguan. The program
did not succeed. Steiner et al. (1970) attribute the failure of this program
to several factors, but principally to the difficulty of maintaining adequate
overflooding ratios because of low longevity of released flies. Mortality
was ascribed to thermal damage incurred by pupae during transport; to
inadequate food sources available to emergent adults; to predation by
toads, poultry, ants, and lizards; and to the failure of emerging adults
to move from release sites into breeding areas. The oriental fruit fly
was subsequently eradicated from these islands by using the method of
male annihilation (Steiner et al. (1970)).

The eradication of the oriental fruit fly from the Mariana Islands
freed this area of fruit flies except on Guam, where the melon fly remained.
From this source, the insect was reintroduced to the nearby island of
Rota on 7 occasions up to January 1969. Each time the development of
extensive populations was curtailed by prompt release of sterile flies
shipped from Honolulu. Usually, releases of one-half to 1 million flies/week
for periods of 9 weeks to 6 months were required to eradicate these small
populations.

Continuous melon fly trap surveys have been conducted on Guam since
July of 1963. The melon fly population dropped to very low levels in the
spring of 1965 and has not yet returned to the high levels observed in the
previous years. The presence of only small numbers of native flies
made possible the implementation of a sterile-release program on Guam.
Accordingly the Department of Agriculture undertook such a program in
1967 with an appropriation of $ 350 000 to be provided over a 3-year
period by the Legislature of the Government of Guam. Neal Spencer is
Project Director. The technical assistance of the Hawaiian Fruit Flies
Investigations, Honolulu, Hawaii, was enlisted, where the design for
the original program and the rearing facility were undertaken by Drs.
L. F. Steiner and R. A. Hart, and the equipment and mass-production
methods were developed by N. Tanaka and T. Kozuma.

For the program, a building of about 400 m^2 located on the grounds
of the Department of Agriculture at Mangilao was completely reconstructed
to provide adequate facilities for rearing and irradiating about 15 million
melon flies/week. It contains rooms equipped with proper lighting, tem-
perature, and humidity control for the various operations, namely:
egging, larval holding, pupal holding, irradiation, sifting and dyeing,
steam-cleaning of equipment, and offices.

In the egging room, the adult flies are held in 30 × 60 × 120-cm
cages of the type described in the review of rearing methods by Nadel
(1970). The cages are stocked with about 25 000 flies each, and the room

holds 64 cages in stacks of 4. The flies are egged 3 times per week and 88 000 of the eggs/tray are seeded onto medium in stackable trays covered to a depth of 1 inch (2.5 cm) with 7 litres of medium. During the first 3 days of development, the larvae are held at 27°C in total darkness to ensure uniform utilization of the diet (with light they tend to concentrate in areas of low intensity). During the final 3 days of the larval period they are held at 21°C to prevent overheating of the medium. Larvae emerge from the medium ad libitum and drop into water in a pan placed at the base of each stack of trays. The immersion in water causes them to become quiescent. Then every 6 - 7 hours they are drawn off into cloth bags and placed in moist (about 5% water) vermiculite, 1 litre of larvae/6 litres of vermiculite. After the mixture of larvae and vermiculite has been tumbled in a concrete mixer to ensure proper distribution, it is apportioned into holding boxes. When puparium formation is complete, the pupae are separated from the vermiculite in a rotating sifter, placed in thin layers in screen-bottomed trays, and held at 20 or 27°C. The holding temperature is manipulated to synchronize adult emergence, as described by Tanaka, Okamoto and Chambers (1969). Forty-eight hours before completion of pupation the insects are placed in canisters (1000 each) and irradiated in a Gammacell 220®cobalt source, where they receive a dose of 10 kR ±10% at a rate of 5250 rad/min. Then they are dyed by tumbling them with dye.

The dyed, irradiated pupae which were to be airdropped as bagged adults were transported to another building and prepared for distribution. The system used, described by Holbrook et al. (1970), utilized #12 paper bags and a cardboard insert. On an assembly line basis, 3000 pupae were placed in each bag with sugar cubes, which sustained the adults after they emerged within the bags. The paper bags were sewn closed and stacked within large cloth bags, in which they were transported to the aircraft when 80-90% of the adults had emerged.

The aircraft utilized to distribute the flies was a DC-3 under contract to the Government of Guam, fitted with a chute designed by the United States Air Force in Panama which extended out of the rear cargo hatchway. The bags were manually dropped into the chute at a rate established by an adjustable flashing light signaller and were drawn down the chute by a Venturi effect, where they were slit by four adjustable knives fitted into the sides of the chute near the bottom exit, allowing the flies to escape when the bag dropped to the ground. Three flights were made each week, each covering a different third of the island in a decreasing spiral pattern.

The first releases of sterile melon flies (a total of 1.7 million) were made the second week of March 1969. Distributions by airplane continued through July 1969, and averaged about 10 million flies per week.

In the middle of August it was necessary to terminate the aerial drops and all the subsequent production was distributed in about 200 ground-release cages. Pupae are released at least once each week in every cage and the number released in a given area is adjusted for differences in recovery rates, native fly population and breeding host abundance. Pupae are distributed at less frequent intervals in remote areas which, in general, are not good breeding host areas and where native fly populations are very low or absent.

We are confident that the melon fly will be eradicated from Guam with the advent of the dry season of winter and spring, when hosts and food sources are scarce and the wild fly population normally is greatly reduced.

Note

Mention of a proprietary product in this paper does not constitute an endorsement of the product by the U.S. Department of Agriculture.

REFERENCES

HOLBROOK, F.R., STEINER, L.F., FUJIMOTO, M.S. (1970). Holding containers for melon flies and Mediterranean fruit flies for use in sterile fly aerial releases, J. econ. Ent. 63, pp. 908-10.

NADEL, D.J. (1970). "Current mass rearing techniques for the Mediterranean fruit fly", these Proceedings.

STEINER, L.F., MITCHELL, W.C., BAUMHOVER, A.H. (1962). Progress of fruit fly control by irradiation sterilization in Hawaii and the Mariana Islands, Int. J. appl. Radiat. Isotopes 13, pp. 427-34.

STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D. (1965a). Melon fly eradication by overflooding with sterile flies, J. econ. Ent. 58, pp. 519-22.

STEINER, L.F., MITCHELL, W.C., HARRIS, E.J., KOZUMA, T.T., FUJIMOTO, M.S. (1965b). Oriental fruit fly eradication by male annihilation, J. econ. Ent. 58, pp. 961-4:

STEINER, L.F., HART, W.C., HARRIS, E.J., CUNNINGHAM, R.T., OHINATA, K., KAMAKAHI, D.C. (1970). Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release, J. econ. Ent. 63, pp. 131-35.

TANAKA, N., OKAMOTO, R., CHAMBERS, D.L. (1970). Methods of mass rearing the Mediterranean fruit fly currently used by the U.S. Department of Agriculture, these Proceedings.

NEW ARTIFICIAL OVIPOSITION DEVICE
FOR THE EUROPEAN CHERRY FRUIT FLY

R. J. PROKOPY, E. F. BOLLER
Eidgenössische Forschungsanstalt
für Obst-, Wein- und Gartenbau,
Wädenswil, Switzerland

Abstract

NEW ARTIFICIAL OVIPOSITION DEVICE FOR THE EUROPEAN CHERRY FRUIT FLY.

A new and highly effective artificial oviposition device (ceresin wax domes) for the European cherry fruit fly, Rhagoletis cerasi L., is described.

Lack of an effective artificial oviposition device has hampered the development of techniques for continuous and large-scale laboratory culturing of the European cherry fruit fly, Rhagoletis cerasi L., an important pest of cherries. A first oviposition device consisted of a small gelatin sphere wrapped in parafilm (Boller (1968)). Although large numbers of eggs were deposited in these spheres, we felt that a better device was needed because egg hatch was only fair at best and the device had to be destroyed and renewed daily. This last statement was also true of the parafilm-covered foam balls developed by Haisch (1968).

Small paraffin - petrolatum - cheesecloth domes and small parafilm-covered wire hemispheres (Prokopy (1966, 1967)), developed as artificial oviposition devices for the apple maggot, R. pomonella (Walsh), were tried. However, the process required to construct sufficient quantities of either device proved too laborious and costly for practical usage on a large scale. Hagen et al. (1963) and Tzanakakis (1967) developed a technique in which the convex end of a glass test-tube was dipped in soapy water and then into molten wax to make wax domes as oviposition devices for the olive fly, Dacus oleae Gmelin. We utilized this latter technique to form a new, highly effective, durable, and mass-producible oviposition device (ceresin wax domes) for the cherry fly.

Wiesmann (1937) described a number of characteristics of the host fruit (shape, size, hardness and smoothness of surface) which he found to be important in eliciting oviposition attempts by R. cerasi females. So our first experiments concerned the optimal shape and size of oviposition device. We found that spheres and hemispheres were much more effective than any other shape tested. Hemispheres oriented with their convex surface up and positioned on or near the cage floor were just as effective as similarly positioned spheres. Hemispheres were less effective, however, when their orientation was reversed or when they were attached to the sides or top of the cage. It is worth noting here that under the conditions of our experiments (the flies exposed to 2800 lux of direct, overhead light from mercury vapour lamps), the flies showed a distinct tendency to spend more and more time on and about the cage floor as

they became older, irrespective of the presence or absence of oviposition devices. Among the various sizes tested, hemispheres (domes) 10 mm in diameter $\times$ 8 mm high proved the most effective.

After establishing the optimal shape, size, and position of the device, we proceeded to test various types and combinations of wax-like materials for making the domes. In all experiments involving the use of various paraffins or beeswax, alone or in combination with vaseline and/or mineral oil, the domes formed were too hard to permit easy ovipositor penetration. The result was that the majority of eggs were not deposited inside the domes but dropped outside. Finally, we located a wax (Type 1577 soft ceresin, manufactured by Deutsche Erodel AG, Mittelweg 180, Hamburg, Germany) which embodied the unique combination of a high melting point (62-66°C) and a high index of penetration or softness (45-55 at 25°C). Cherry fly females were able to penetrate these ceresin domes without any difficulty. Thus, more than 90% of the eggs were deposited inside domes 0.2 mm thick, with domes of this thickness perfectly retaining their hemispherical shape and remarkably smooth surface even at temperatures far above the 25°C in the rearing room. Dilution of the ceresin wax with vaseline or mineral oil resulted in decreased effectiveness and durability compared with domes formed from 100% ceresin.

Further experiments were carried out to determine the best colour of dome and the most favourable conditions at the inner and outer dome surface. Black domes (0.3 g of powdered black candle wax dye per 100 ml of ceresin) against a white background proved to be as effective as any other colour-contrast system tested. This system was adopted as standard because the black dye remained stable and did not fade in colour as did blue, green, yellow, orange and red-dyed domes after a few days' exposure to the rearing room lights. A system using white domes against a black or white background was the least productive of those tested. Domes having a rough outer surface were less effective than those with a smooth outer surface. If the humidity in the cages was increased from its normal level of 75% to 95% or more, a thin film of moisture condensed to cover the outer dome surface and females stopped ovipositing. Fewer eggs were deposited when the inner dome surface was wet than when it was moist or dry. Addition to the inside of the domes of fresh cherry leaves, pedicels, or fruit in optimum stage for oviposition had no influence on oviposition.

On the basis of these findings, the following type of oviposition device was adopted as standard: domes constructed of 100% Type 1577 ceresin wax, 10 mm diameter $\times$ 8 mm high $\times$ 0.2 mm thick, black in colour and on the cage floor against a white background, smooth outer surface, and a piece of moist cotton inside to prevent egg desiccation. Almost without exception, eggs deposited inside the domes remained lightly attached to the inner dome surface. They were easily removed without injury either to eggs or domes by a gentle, whirling stream of water from a squeeze-type, plastic wash bottle.

When 50 females and 30 males were caged with 36 of these standard domes and fed a diet of raw brown sugar and enzymatic yeast hydrolysate, 12 903 eggs were deposited into the domes, about 91% of which hatched. Oviposition began on the 4th day, continued at a high level for 5 weeks, and ceased after 7 weeks. In another experiment, we found that individual

domes retained 100% of their maximum potential effectiveness after receiving 50 eggs each, and 68% of their potential even after 200 eggs each. Future work will concern the optimal number of females and domes per cage and whether or not the domes must be renewed for maximum economy and efficiency in the system.

A simple technique for mass production and employment of plates of large numbers of these domes was developed. An account of this technique, as well as a complete presentation of data on the comparative effectiveness of all the different types of oviposition devices and systems tested is given elsewhere (Prokopy and Boller (1970)).

REFERENCES

BOLLER, E. F. (1968). An artificial oviposition device for the European cherry fruit fly, Rhagoletis cerasi, J. econ. Ent. 61, pp. 850-2.

HAGEN, K. S., SANTAS, L. TSECOURAS, A. (1963). "A technique of culturing the olive fly, Dacus oleae Gmel., on synthetic media under xenic conditions", Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963) IAEA, Vienna (1963) pp. 333-56.

HAISCH, A. (1968). "Preliminary results in rearing the cherry fruit fly (Rhagoletis cerasi L.) on semi-synthetic medium", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests (Proc. Panel Tel Aviv, 1966) IAEA, Vienna (1968) pp. 69-78.

PROKOPY, R. J. (1966). Artificial oviposition devices for apple maggot, J. econ. Ent. 59, pp. 231-2.

PROKOPY, R. J. (1967). Factors influencing effectiveness of artificial oviposition devices for apple maggot, J. econ. Ent. 60, pp. 950-5.

PROKOPY, R. J., BOLLER, E. F., (1970). Artificial egging system for European cherry fruit fly, J. econ. Ent. 63, pp. 1413-7.

TZANAKAKIS, M. E. (1967). Control of the olive fruit fly, Dacus oleae (Gmelin), with radiation or chemical sterilization procedures (unpublished report).

WIESMANN, R. (1937). Die Orientierung der Kirschfliege, Rhagoletis cerasi L., bei der Eiablage, Landw. Jb. Schweiz 51, pp. 1080-1109.

REARING EXPERIMENTS WITH THE EUROPEAN CHERRY FRUIT FLY

A. HAISCH

Bayerische Landesanstalt für Bodenkultur,

Pflanzenbau und Pflanzenschutz,

Munich, Federal Republic of Germany

Abstract

REARING EXPERIMENTS WITH THE EUROPEAN CHERRY FRUIT FLY.

Experiments have shown that the collection of eggs of Rhagoletis cerasi L. with artificial egging devices is possible, and the method is adequate for the present stage of the work. The main problems in rearing the larvae are not only due to the difficulty in developing an adequate feeding mixture but also in applying effective but non-toxic means of preservation.

For several years experiments have been carried out to rear the European cherry fly under laboratory conditions. This short paper describes the investigations.

EGG COLLECTION

The eggs were collected by different methods. The females had the possibility of laying their eggs in grapes or in red-coloured agar-agar balls (2 cm ⌀), or of dropping them through the meshes of their gauze cage on a wet filter paper. In a separate experiment the eggs were gathered by a method developed by Prokopy, using domes made of a black wax, similar to those which Hagen had described, but much smaller. The egg production and the egg fertility with the different egg collection methods are shown in Table I. The technique of Prokopy turned out to be superior to the others, since it resulted in the highest number of larvae per female.

LARVAL FOOD

The main problem is how to feed the larvae. Table II shows most of the food components tested and the various concentrations. The quality of the food mixtures was judged according to the time that the larvae needed to reach the different development stages when feeding on the mixture.

Important but not decisive is the consistency of the food mixture. At the early larval stages there is the danger of being drowned because the water content of the medium is still high and the larvae are small. Later the medium becomes dry and therefore perhaps too hard for the larvae. Although wheat bran and peat can be used for mixtures to feed larvae of the Mediterranean fruit fly, the larvae of the cherry fruit fly did not grow on such mixtures. Cellulose with a concentration of more than 15% was also detrimental because the mixtures dried too quickly. In the mixture of

TABLE I. COMPARISON OF DIFFERENT KINDS OF EGG COLLECTION

Experi- ment No.	Kind of egg collection	Eggs per female		Egg fertility % ± s*
		Absolute	Relative	
1	Dropping	61.3	84.5	6.5 ± 7.3
1	Agar-agar balls	2.7	3.7	52.5 ± 16.8
1	Grapes	8.5	11.8	41.2 ± 17.1
1	Total:	72.5 ± 8.2	100.0	--
2	Wax domes	66.6 ± 11.2	--	85.7 ± 25.9

* Deviation from the regression line (regression of number of hatched larvae on number of eggs).

TABLE II. FOOD COMPONENTS AND THEIR CONCENTRATIONS USED IN THE REARING EXPERIMENTS

Ingredient	Concentration tested (%)	Concentration (%) of the best mixture
Agar-agar	1	-
Peat	0 - 17.6	-
Wheat bran	0 - 40.0	-
Wheat germ diet [a, b]	0 - 40.0	10.4
Cellulose [c]	0 - 40.0	13.4
Powdered carrots	0 - 8.0	4
Dried brewer's yeast	4.0, 8.0	4
Sugar	4.0	4
Vitamin fortification mixture [b]	0 - 4.8	2.4
ß-carotene	0, 0.0012, 0.0024	-
Wesson's salt mixture [b]	0 - 1.5	-
Water	44 - 80	44
Hydrochloric acid (10%)	0 - 1.2	-
Formalin	0 - 0.04	0.04
Benzoic acid	0 - 0.05	0.05
Sorbic acid	0 - 0.02	-
Nipagin M	0 - 0.10	0.10
Nipasol	0 - 0.10	-
Pimaricin [d]	0 - 0.004	-
pH	4.1 - 4.7	4.5

[a] Wheat germ diet consists of casein (28%), sucrose (28%), wheat germ (24%) salt mixture (8%) and alphacel (12%).
[b] Nutritional Biochemical Corporation, Cleveland, Ohio, USA.
[c] Schleicher and Schüll, Kassel, Federal Republic of Germany.
[d] Basotherm GmbH, Biberach, Federal Republic of Germany.

cellulose, powdered carrots and Vanderzant's wheat germ diet, the larvae could bore sufficiently during their whole development. But these substances contain also food components, so that it is difficult to judge its specific quality as carrier substance. For instance, 4% powdered carrots in the medium definitely accelerated the growth and lowered the mortality of the larvae. It could not yet be demonstrated that this fact is due to the carrotene content of the carrots, an assumption which was made because of an observation of Monro[1], who has fed larvae of the Mediterranean fruit fly. The growth of the larvae was also accelerated after the yeast content was increased from 4 to 6 and 8%. However, the mortality at the beginning of the 2nd instar was also increased. The effect of an additional vitamin mixture, Wesson's salt mixture or the trace minerals zinc and cobalt was tested, but because the results were negative, no conclusions could be drawn.

It is very important for the good development of larvae that the growth of micro-organisms be adequately prevented. Most of the food mixtures tested were very heavily contaminated with yeast, which covers the food with a viscous substance. Several experiments showed that formalin or benzoic acid with Nipagin M or Nipasol did not control the yeast. Potassium sulphite (0.1%) prevented the growth of micro-organisms and larvae. Sorbic acid (0.02%) was effective against the yeast and also toxic for the larvae, but less toxic to the larvae than potassium sulphite. Pimaricin killed all micro-organisms but the growth of the larvae seemed also to be retarded. Only a few larvae survived to the beginning of the 2nd instar.

The best growth of the larvae was observed with the mixture shown in the right-hand column of Table II. The shortest time which the larvae needed to reach the 2nd instar was 3 days, to reach the 3rd instar 9 days, and to pupate 16 days. The mortality was roughly 90%. The rate of recovery could be markedly higher if the contamination by yeasts could be prevented.

Tests being carried out will show whether the harmful effect of Pimaricin on larval development is caused by the killing of the symbionts, which may enable the larvae to digest proteins. In this case the substitution of protein by its hydrolysed products would essentially improve the growth of larvae.

[1] MONRO, J., in Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests, IAEA, Vienna (1968) pp. 91-104.

.

STERILE-MALE TECHNIQUE FOR ERADICATION OF THE MEXICAN AND CARIBBEAN FRUIT FLIES
Review of current status

FERNANDO LOPEZ D.
Entomology Research Division,
Agricultural Research Service,
United States Department of Agriculture,
Miami, Florida, United States of America

Abstract

STERILE-MALE TECHNIQUE FOR ERADICATION OF THE MEXICAN AND CARIBBEAN FRUIT FLIES: REVIEW OF CURRENT STATUS.

Research at the Mexican Fruit Fly Investigations laboratory in Mexico City, Mexico, with the Mexican fruit fly *Anastrepha ludens* (Loew) demonstrated that this fly could be artificially reared in a carrot-base medium and sterilized with 5000 rad. Adult flies could also be sterilized by dipping them as pupae in a 5% solution of tepa in water. Field fly releases with either sterilization method demonstrated that the sterile-fly release method is useful for control purposes or possible eradication if the infested area can be overflooded with sterile flies.

A cottonseed hydrolysate-borax solution exposed as a lure for the Mexican fruit fly in invaginated glass traps proved to be superior to PIB-7 — borax (the standard). This new lure in a pellet form, placed in water in the glass traps, improved trapping procedures.

Studies on the Caribbean fruit fly *Anastrepha suspensa* (Loew) carried out in Miami, Florida, since 1968 are giving encouraging results for the application of the sterile-male technique. Flies are being reared in several types of media, although at present the hatch rate and larval recoveries are low. Radiation doses required to sterilize this fly are in the range of 5000 to 6000 rad.

A Torula yeast hydrolysate at 3% plus 4% borax proved to be about 5 times as attractive as the standard cottonseed hydrolysate-borax pellets used as a standard in the detection program in Florida. No significant differences were found when this Torula yeast-borax formulation was used in a pellet form.

Aerial application of a bait spray containing 280 g PIB-7 and 70 g technical malathion applied as large droplets was more effective than 70 g of technical malathion applied as an ultra-low volume spray.

MEXICAN FRUIT FLY

The Mexican fruit fly, *Anastrepha ludens* (Loew), one of the most important species of the genus *Anastrepha*, breeds in wild and cultivated citrus in north-eastern Mexico and is a constant threat to similar fruits in California, Arizona and Texas.

The United States Department of Agriculture established the Mexican Fruit Fly Investigations laboratory in Mexico in 1928 in co-operation with the Secretaria de Agricultura y Fomento, to study the biology, ecology and methods for detection and control of this fly.

From the beginning of this program until the development of the sterile-male technique, several entomologists and chemists under the direction of A.C. Baker et al. (1944) investigated, among other things, the nature of the damage caused by the fly, host preferences, biology of the larvae, pupae and adults, trapping procedures, control of the adults by sprays, parasites and diseases and disinfestation of fruit by fumication and other

treatments. Bait sprays proved to be a successful method of controlling the fly population, but held several disadvantages.

Studies were initiated to find more advantageous methods to prevent establishment of this fly in the U. S. Results of this research indicated that the sterilization method was superior to bait sprays for reducing populations of the Mexican fruit fly. We therefore undertook a program to mass-rear the fly for sterile-male releases. In 1954, Hagen and Lopez (unpublished data, Mexican Fruit Fly Investigations, Mexico, D. F.) initially reared the Mexican fruit fly on a fresh carrot medium modified from an early Hawaiian formula. The subsequent development of an oviposition receptacle by McPhail and Eguiza (1956) increased the efficiency of production methods. Later Rhode and Spishakoff (1964), on the basis of results obtained by Christenson et al. (1956), substituted dehydrated powder and granules of carrot for fresh carrot in the medium which improved the mass-rearing technique. The formulation of this medium was as follows:

Water	80.92%
HCl	0.60%
Sodium benzoate	0.08%
[1]Tegosept®	0.10%
Brewer's yeast	4.00%
Yeast hydrolysate	0.30%
Dehydrated carrot (granulated)	7.00%
Dehydrated carrot (powder)	7.00%

In the Monterrey facility at Monterrey, Mexico, the mass production of flies and sterilization is conducted by J. West and L. M. Spishakoff. The flies are reared in a medium in which corn-cob grits were substituted for the granulated carrot, the powdered carrots were retained, and sugar was added.

This new formulation is as follows:

Water	70%
HCl	0.6%
Sodium benzoate	0.08%
Tegosept®	0.1%
[2]Torula yeast CF 2	5%
Sugar	6.22%
Corn-cob grits	13%
Powdered carrots	5%

---

[1] Butyl p-hydroxybenzoate.
[2] Lake States Division, St. Regis, Rhinelander, Wisconsin, USA.

About 50% of the eggs set on this medium produce larvae for transfer to the pupation medium. The weekly production is 1.5 million pupae.

When the techniques of mass production were well established, Rhode et al. (1961) began in 1958 to investigate irradiation dosages and effects using the pupal stage which lasts about 15 to 16 days. They found that when 4-day-old pupae were treated with more than 2000 rad no adult emergence was obtained. Eight-day old pupae treated with 7000 to 12 000 rad gave 20% to 60% lower emergence of adults than the untreated. With pupae 12 days old, or about 4 days before adult emergence, radiation up to 12 000 rad did not affect the emergence and complete sterility was obtained for both sexes at the 5000 rad level.

The dose rate was investigated at 10 R, 30 R, 50 R, 70 R and 90 R per minute for the radiation doses from 1000 to 5000 with increments of 1000 R. At 2000 rad, with a dose rate of 70 or 90 R per minute, females laid fewer eggs and males became less fertile than normal flies. The females treated with 3000 or more rad developed no eggs and the number of viable eggs deposited by untreated females mated with treated males gradually diminished until complete sterility was obtained at 5000 rad.

In two overflooding tests in the laboratory, a rate of 50 sterile flies to 1 native reduced the percent hatch from 74.1 and 85.5% to 0 and 0.7% respectively.

To test the effectiveness of this method under natural conditions, ground field releases of sterile flies were made at two semi-isolated locations in Mexico. The results showed that a ratio of 66 sterile to 1 native fly reduced larval infestation in mangos from about 30 larvae/kg to the unusually low level of 0.81 larvae/kg. However, when the native populations were high and only a ratio of 8:1 was obtained, larval infestations were similar to untreated plots.

Native flies are brought into the Tijuana area annually in infested fruit and constitute a continued threat of establishment and spread into fruit-growing areas of the South-west.

The USDA Plant Pest Control Division's facility at Monterrey, Mexico, initially under the direction of L.F. Curl (at present under J.S. Parker) is used for the sterilization of Mexican fruit flies with a gamma irradiator. The Monterrey facility now provides the gamma-irradiated flies for release along the border to prevent establishment by migrants into California.

Pupae treated with 6000 rad are placed in ground-release stations in Tijuana and La Paz, B.C., when the fly is detected by trapping. Flies in the ground-release stations emerge through a layer of vermiculite dyed with calco blue. They can be differentiated from the native population when the flies are crushed with a glass rod previously dipped in acetone (Steiner (1965)).

In the Tijuana area, native populations are kept under control by using this method. In La Paz, B.C., an isolated location with a low native population, it appears that the population was eradicated, since no wild flies were detected during the last 6 months (Spishakoff, personal communication).

During the period from 1959 to 1961 research on gamma sterilization was suspended and studies on chemosterilants were initiated in 1962 by Shaw and Sanchez (1965). They found that after pupae were dipped in a 5% aqueous solution of tepa for 1 minute and allowed to dry for 24 hours, the flies were sterilized when the adults emerged and touched the residue on the exterior puparia. When such tepa-sterilized flies were released in a semi-isolated area in Mexico, in numbers sufficient to overflood the native populations (300-

500 sterile flies to 1 wild fly), 12 to 14% of the mango crop harvested was infested in the release grove as compared to 89-90% infestation in the untreated grove. The same authors demonstrated that adults of the Mexican fruit fly could also be sterilized by drinking water with 0.025% tepa. Bait stations with the chemosterilant (Sanchez and Shaw (1966)) were exposed in a 1-acre planting of 32 mango trees and the infestation of Mexican fruit flies was decreased in the test area as compared to the check. Because of lack of replication and different ecological conditions, no significant differences were found.

However, in 1963, releases of tepa-sterilized males (Shaw et al. 1966, 1967) were made at Tijuana and along the border in co-operative tests with Mexican, USDA and California officials, and bait sprays were discontinued. High overflooding rates (500 to 1000 sterile male flies to 1 wild male fly) apparently prevented establishment of infestations although some wild flies (possibly migrants) were captured in survey traps. The dosage of chemosterilant could not be controlled and sterilization of females could thus not be guaranteed. In 1966, flies of both sexes, sterilized with gamma irradiation, were substituted for the tepa-sterilized flies.

Since the establishment of the laboratory in Mexico, studies on survey methods have been made by several investigators. McPhail (1937) found that the invaginated glass trap was the most effective for liquid lures. He investigated the fermenting sugar lures as well as proteinaceous substances and found them to be generally attractive to A. ludens. In 1955, J. F. Cooper and associates (unpublished data, Mexican Fruit Fly Investigations, Mexico, D. F.) worked with acid, basic and enzymatic hydrolysates of proteins, but these were not as attractive as the standard fermenting lure, and in addition, they putrefied with age. After screening a large number of chemicals it was found that an acid hydrolysate of corn protein with a corn-steep water (known as Staley's Insect Bait No. 7 [now PIB-7]),[3] proved to be the most stable and efficient attractant for the Mexican fruit fly. This lure was used as standard for several years but some of the disadvantages were that after several days of exposure, decomposition and discoloration were present, soft bodies such as fruit flies disintegrated, and extraneous insects were attracted. Lopez and Hernandez (1967) found that the addition of sodium borate eliminated putrefication and the other disadvantages.

Subsequently a more effective lure was developed. Lopez and Spishakoff (1963 a, b) found that a solution prepared with 1% enzymatic cottonseed hydrolysate[4] and 2% borax was more attractive than the PIB-7 borax solution. The liquid hydrolysate-borax bait was replaced in 1961 by a pelletized formulation of an enzymatic cottonseed hydrolysate and 2.6% borax and is the standard for Mexican fruit-fly detection in the control program carried out in the Mexican-U. S. border area. It is also one of the detection tools for foreign tephritids used by regulatory officials in several other parts of the United States.

Before the Mexican fruit fly investigations were terminated in 1968, more than 11 000 compounds had been screened as attractants. Unfortunately, it never was possible to obtain as powerful a male lure as the methyl eugenol, trimedlure and cue-lure developed for the three Hawaiian fruit fly species.

---

[3] A. E. Staley Manufacturing Co., Decatur, Ill., USA.
[4] Amber Laboratories, Milwaukee, Wis., USA.

CARIBBEAN FRUIT FLY

The Caribbean fruit fly, Anastrepha suspensa (Loew), invaded Florida in 1965 and caused considerable economic damage. It spread northward through 28 counties. The U. S. Department of Agriculture, Entomology Research Division, established a Caribbean Fruit Fly Investigations at Miami, Florida, under the direction of L. F. Steiner in 1968. Some work had been conducted before 1968 by the USDA and the Florida Department of Agriculture.

H. Kamasaki and associates in 1965 found that the Mexican fruit fly media could be used to rear this fly, but less successfully than A. ludens. When the colony was in the F_3 generation it was turned over to the Division of Plant Industry, Florida Department of Agriculture, which successfully carried it to the 7th generation.

Richard Baranowski of the University of Florida Subtropical Experiment Station at Homestead, Florida, and A. Selhime and associates at the USDA Humid Area Insects Research Laboratory at Orlando, Florida, continued with research to improve fly production. They found that the sugar cane – bagasse media used in Costa Rica for the Mediterranean fruit fly eradication program could be used with some modification such as with the addition of citrus pulp. However, productivity was low and the methods require further improvement before efficient mass production can be obtained.

Paul Norman and associates established the Orlando colony at Miami, Florida, and used the carrot type medium as a standard on a mass-production basis and compared it with a new Hawaiian medium developed by N. Tanaka and associates (unpublished manuscript, Hawaiian Fruit Flies Investigations laboratory) and the previously mentioned bagasse – citrus pulp media. The recoveries obtained with the last two media were generally lower than obtained with the carrot medium. However, at present the percent larvae recovered for transfer to a pupation medium from eggs set with any of these media rarely exceed 15%, partly because of low fertility (averaging 55%). The current mass-production studies need further evaluation before any large field tests of the sterile-insect release method can be conducted. Studies by Kamasaki and associates in 1965 showed that the radiation dose needed to induce sterility was about 3000 rad. They found it could be increased to 5000 rad without adversely affecting the behaviour of the adult.

Studies were initiated at Miami, Florida, in 1968 on radiation dosages and their effects but little of this has been completed. To judge from dissections made of treated flies, the dose required to obtain irreversible sterility will be in the range of 5000-6000 rad. However, much more research must be done to determine the minimum effective doses that can be used for fly releases and the overflooding ratios required.

Research on attractants for the Caribbean fruit fly showed that the pelletized enzymatic cottonseed hydrolysate and borax in invaginated glass traps with water was also attractive for this fly as well as the Mexican fruit fly. This formula was used for survey purposes to detect Caribbean fruit fly populations.

Early in the attractant studies Lopez and associates at the Miami laboratory found that a hydrolysed Torula yeast[5] at 3% plus 4% borax in

---

[5] Amber Laboratories, Milwaukee, Wis., USA.

water proved to be 5 times as effective in invaginated glass traps as the standard pelletized cottonseed hydrolysate-borax lure.

We tested pellets prepared with this new lure by the USDA Plant Pest Control Division, at the same concentration in water in the invaginated glass traps replicated 50 times. The pelletized bait caught about 15% less flies than the Torula - borax solution. But the analysis of variance and Duncan multiple range test showed no significant difference. Current tests with higher ratios of hydrolysate and borax than 1:1.33 indicated that more than 2 parts borax to 1 of the hydrolysate is harmful. The borax reduces the attraction most during the first 2 days.

Olfactometer screening of the candidate materials are under way with the purpose of finding a strong male or female attractant that can be used to greatly improve present detection and survey procedures. Only two chemicals of 464 screened to date showed any attraction but not enough for potential use. However, these may provide USDA chemists with valuable leads in our mutual search for better attractants.

F.R. Holbrook and associates at the Miami laboratory in co-operation with USDA Plant Pest Control Division, University of Florida, and the Division of Plant Industry (Florida State Department of Agriculture) conducted replicated aerial bait spray tests on sixteen 145-hectare plots in the city of Miami to compare three concentrations of PIB-7 — malathion with ultra-low volume malathion for suppression of the native population. The object was to develop dosages that could be used before sterile releases. They found that a mixture containing 112 g of PIB-7 and 28 g of technical malathion/ha applied as large droplets was not statistically different from 28 g of technical malathion alone applied as a ULV spray, although the average reduction during the week after each spray was 88% and 57% for the bait and non-bait sprays, respectively.

Note

Mention of a commercial product or company does not necessarily imply their endorsement by the USDA.

REFERENCES

BAKER, A.C., STONE, N.E., PLUMMER, C.C., McPHAIL, M. (1944). A Review of Studies on the Mexican Fruit Fly and Related Mexican Species. US Department of Agriculture Miscellaneous Publication No.531, Washington, D.C.

CHRISTENSON, L.D., MAEDA, S., HOLLOWAY, J.R. (1956). Substitution of dehydrated for fresh carrots in medium for rearing fruit flies, J. econ. Ent. 49, pp.135-6.

LOPEZ-D., F., HERNANDEZ-B., O. (1967). Sodium borate inhibits decomposition of two protein hydrolysates attractive to the Mexican fruit fly, J. econ. Ent. 60, pp.137-40.

LOPEZ-D., F., SPISHAKOFF, L.M. (1963a). Reaccion de la mosca de la fruta Anastrepha ludens (Loew) a atrayentes proteicos y fermentables, Ciencia (Mexico) 22 (4), pp.113-4.

LOPEZ-D, F., SPISHAKOFF, L.M. (1963b). Finding new lures for Mexican fruit fly, Calif. Citrogr. 48 (10), pp.354-7.

McPHAIL, M. (1937). Relation of time of day, temperature and evaporation to attractiveness of fermenting sugar solution to Mexican fruit fly, J. econ. Ent. 30, pp.793-9.

McPHAIL, M., EGUIZA, F. (1956). An oviposition medium for the Mexican fruit fly A. ludens (Loew). J. econ. Ent. 49, p.570.

RHODE, R.H., LOPEZ-D., F., EGUIZA, F., TELICH, J. (1961). Effect of gamma radiation on the reproductive potential of the Mexican fruit fly, J. econ. Ent. 54, pp.202-3.

RHODE, R.H., SPISHAKOFF, L.M. (1964). Tecaicas Usadas en el Cultivo de Anastrepha ludens (Loew). Il Memoria del Dia del Parasitologo. Secretaria de Agr. y Granderia. E.M.A. pp.28-8.

SANCHEZ-R., M., SHAW, J.G. (1966). Use of bait stations in chemosterilant control of the Mexican fruit fly, J. econ. Ent. 59, pp.753-4.

SHAW, J.G., SANCHEZ-R., M. (1965). Effectiveness of tepa-sterilized Mexican fruit flies released in mango grove, J. econ. Ent. 58, pp.26-8.

SHAW, J.G., PATTON, W.P., SANCHEZ-R., M., SPISHAKOFF, L.M., REED, B.C. (1966). Mexican fruit fly control, Calif. Citrogr. 51 (5), pp.209-14.

SHAW, J.G., SANCHEZ-R., M., SPISHAKOFF, L.M., TRUJILLO, G.P., LOPEZ-D., F. (1967). Dispersal and migration of tepa-sterilized Mexican fruit flies, J. econ. Ent. 60, pp.992-4.

STEINER, L.F. (1965). A rapid method for identifying dye-marked fruit flies, J. econ. Ent. 58 (2), pp.374-5.

STERILE-MALE TECHNIQUE FOR CONTROL OF THE OLIVE FLY

Review of work on rearing and radiation

G. de MAGALHÃES SILVA
Estração Agronómica Nacional,
Oeiras, Portugal

Abstract

STERILE-MALE TECHNIQUE FOR CONTROL OF THE OLIVE FLY: REVIEW OF WORK ON REARING AND RADIATION.

All aspects of rearing Dacus oleae (Gmelin) are reviewed. Results indicate that the composition of the diet is quite satisfactory and that the main problems are adequate egg-laying devices as well as reduction in rearing space. Work on radiation and chemical sterilization is mentioned. Larvae, pupae and adults were sterilized. As expected, a higher dose was needed for the more advanced phase. Different authors report work on male competitiveness and on field cage tests. Tepa seems to be the most promising of the various chemosterilants tested.

1. INTRODUCTION

One of the insects whose activity as a pest has been referred to over a very long period (Roman authors mentioned it) is the olive fly, Dacus oleae (Gmelin). In its biology it presents very marked characteristics which are not very common among fruit flies: it is strictly monophagous; it lays only one egg at a time and, conditions permitting, one in each fruit.

As a matter of fact, only fruits of the genus Olea are considered as a host by this species and, on the other hand, where more than one egg is found in each fruit, this happens only during high infestations, when the urge to oviposit, having exhausted all fruits, leaves no other alternative for the heavy egged females but to probe and use already infested fruits.

These characteristics, so deeply marked, become very unfavourable when an artificial substrate is required for laboratory rearing, and mainly when mass rearing is needed. They are responsible, we think, for the difficulties in mass rearing as found by research workers over the last decade, following Moore's first efforts (1959).

2. REARING

Handling of adults

Cages

The cage models used have been mostly of the conventional type, cubic in form with a screen on all sides or with glass in some of them (Hagen et al.(1963), Moore and Navon (1965), Orphanidis et al.(1969), Pelekassis and Santas (1969), Rey (1969)), the size varying from 15-20 cm edge to 35 cm and no one definitely proving which is the best.

Small cage size seems to be essential with this fly, a fact not very encouraging for the economy of mass production.

Multistoried cages of three or four units separated by perforated metal sheets are now used by Silva; they are three or four times lighter than previous cages and cheaper. A horizontal cage of several units, with partial separation, was tried by Silva and colleagues at Oeiras, Portugal, allowing the flies to intermix but keeping them uniformly distributed; this was effective only when the light was on the front side. All these models can be conveniently disassembled for washing and disinfection after use.

Cavalloro (1967) introduced a very cheap cage, made completely of cloth suspended from an individual frame. Silva used a Dexion structure as a frame, providing the sustaining elastic ribbons with small hooks fixed in the holes of the angles.

Tzanakakis (1969) is now experimenting with a larger (1.20 m long) Plexiglas cage with the screen on the underside.

Density of population

The important question of density of population has not yet been completely cleared up, but small densities (Orphanidis et al.(1969), Pelekassis and Santas (1969), Rey (1969), Tzanakakis and Economopoulos (1967)) cannot be ignored if high yields are to be expected with no heavy mortalities.

The findings of Tzanakakis et al. (1968) are important: they point out that the 1:5 (σ : $\mathcal{P}$) ratio is as good as 1:1, and also put forward the possibility of removing the males after 10 days, since from then on, further matings are neither necessary nor to be expected.

Feeding, watering

Up to now, the formula of Tzanakakis et al.(1968), which is basically that of Hagen et al.(1963) enriched with egg yolk and honey, has shown the best results. Olive juice proved unsatisfactory. Cavalloro (1967) claimed to get good results with a solid formula (4:2:1, sugar, yeast, hydrolisate); but Hagen et al.(1963) reported that this kind of food was not adequate for this fly. Tzanakakis et al.(1967c) also tried a solid diet with slightly inferior results but found that a strain of flies from a region with a drier climate was able to use it better: he suggests selection in this direction, taking into account the practical advantages of a solid diet. Rey's (1969) recent claim that his wet formula keeps fluid for a period of 3 weeks, through using 20% glycerine in the water of Hagen's modified formula, could help solve the problem of the need to reduce the routine work. Sugar in powder form is also used by practically all researchers. Water has been provided in different ways: inverted tubes over filter paper in the bottom of cages (Tzanakakis), moistened cotton wicks on the screen top of the cage (Cavalloro), or a water-filled tube inside the cage.

Ambiental factors: light, temperature, humidity

Very different light conditions can be found in various rearing facilities. Basically, there must be a general illumination apparatus and a special lamp, placed over the cages or in front close to the cages. All researchers

agreed that high intensity (1500-3000 lux) is necessary. It seems to the
present author, however, when using Parafilm as an egg collecting device,
that such a great light intensity is not needed, perhaps because of the
translucid property of the film and the habit of the flies to stay on it almost
permanently. 400-700 lux have been used with fair success, either with a
close fluorescent 40-W lamp or with two 60-W lamps, 1m apart. It looks
as if it is not so much the light but the brightness that counts in this case.
Before and after the intense light period, a period of 1-2 hours of reduced
light, reproducing dusk conditions, is sometimes used. The intense light
period ranges from 10 hours (Rey (1969)) to 16-18 hours (Orphanidis et al.
(1969)).

A temperature of 25°C has been adopted almost universally for the
maintenance of adults (Orphanidis et al.(1969), Rey (1969), present author).

Rey, however, uses a period of 20°C during the night; this lengthens
the life span and favours the egg output. Relative humidity is generally
kept at 65-75%.

Oviposition devices

Paraffin domes were introduced by Moore (1959). Either white or
coloured (Orphanidis et al.(1969)),small or somewhat larger, they have
been used by almost all investigators up to now. Multiple, small re-usable
domes were recently tried out by Rey (1969).

Cavalloro's cage (1967), all nylon, provided the opportunity for flies
to oviposit through the fabric, with the eggs falling into trays filled with
water below. The simplicity of this solution, however, resulted in a low .
fecundity of the eggs. Cavalloro's claim of satisfactory fecundity was
unfortunately not confirmed by other investigators (Tzanakakis, Silva).
It seems that when laying, in order to ensure the fecundation of eggs in all
cases, the fly needs to make an effort to puncture some membrane,
adopting the natural position used on the olive fruit, with the abdomen
curved and the ovipositor projected frontwards, so pressing the
spermathecae.

Tzanakakis (1969) and colleagues are also experimenting with a
similar device: the above-mentioned Plexiglas cage with the bottom
screen over a water-filled tray.

Silva has introduced the Parafilm membrane, covering one side of
the cage entirely. This has proved successful, after previous stretching
to 1.5 times the length, reducing the commercially produced thickness
from 100 μm to 67 μm. Upon our request, Marathon Division of Conti-
nental Can Co. has produced a special thinner film which makes this
operation unnecessary.

The females were observed to oviposit readily through the film. How-
ever, as the eggs remained stuck to the outer side of the film, a method
of removing them was needed. To do this, a flushing device was adopted
consisting of a small intermittent syphon delivering $\frac{1}{4}$litre of water every
15-20 min, through a multi-holed plastic tube placed cross-wise on the
upper side of the film. The water, carrying the eggs, was filtered through
a funnel with a close-mesh cloth.

It was found at Oeiras, Portugal, that the pre-oviposition period is
shorter than with domes. This may be related to the difference found by
Rey (1969): in the domes oviposition starts only when 2-3 layers of eggs

are completed, whereas in the fruit only one layer is sufficient. Taking
into account the similarity of physical conditions between both pellicules,
this may be the case, and calls for further verification.

Fertility of eggs was found at Oeiras to be approximately 85% (92-78%)
during four weeks. Some modifications, all cost-reducing, were intro-
duced and now, with the last improved set-up, four 4-storied cages can
be watered with only one syphon and all eggs recovered with only one funnel
at the end of a trough, receiving the water from all the four cages.

Transfer and counting of eggs

The transfer of eggs is done by a paint brush (Rey (1969)), a drop
counter (Silva) or filter paper (Silva), depending on the diet and type of
container used for rearing. For experiments, they are counted indi-
vidually, but for larger rearing containers, pipettes (Cavalloro (1967),
Hagen et al.(1963)) or syringes (Silva) are used as measuring devices.

Immediate transfer after collection is usually considered inadvisable,
with higher mortalities (Orphanidis et al.(1969), Tzanakakis et al.(1966a,
1967b)). Some researchers transfer only larvae (Orphanidis et al.(1969)).
The problem does not seem so acute with diets including corncob (Silva).

Larval substrate

As in all artificial substrates, under non-aseptic conditions, the
following components have to be considered (Friend (1968)): a carrier
or bulk part, mostly inert; protein; carbohydrates; lipids (and steroids);
vitamins; mineral salts; preservatives (mould, yeast and bacteria in-
hibitors). Moore's diet (1959) has evolved to what can be considered now
the least expensive, for a fair yield of pupae: the Rey formula modified
with corncob (1969).

A number of modifications have been made by various authors;
they will be reported here for their historical interest, mainly because
the successes and failures are worth presenting in a discussion on the
lines of research to be followed to reach the final aim of a completely
adequate and inexpensive diet for the olive fly larvae.

Carrier

The bulk of the diet was provided by Moore (1959) as cellulose.
Dehydrated carrot was adopted by Hagen et al.(1963) and Lopez D.
(1965) and is still used by Orphanidis et al.(1969) and Cavalloro (1967).
Other materials have been tried either in a search for a more adequate
physical condition or for less expense, or both: cellulose (Tzanakakis),
bran, either rice (Santas) or wheat (Silva), eucalyptus pulp (Rey), sawdust
(Santas and Pelekassis), ground corncob (Cavalloro). This latter has been
adopted by Silva and Rey and shows the greatest advantages at the moment:
it is very absorbent to water, making the substrate much less dependent
on a very defined amount of water than, for example, cellulose and de-
hydrated carrot (Tzanakakis et al.(1966a)); it gives a fluffy and aerated
condition to the substrate and allows the eggs to be added in a 'sandwich'
fashion (Cavalloro (1967)) or even at the bottom (Silva). As for the cost,
it is only the grinding that has to be considered, since corncob is usually

either thrown away, used for mixing with fertilizers, or burnt. It should
be emphasized that it is not completely without nutritive value, containing
a 40% carbohydrate fraction.

Rey points out that it is somewhat more infected by moulds. When
used in a modification of Tzanakakis's diet P (Tzanakakis et al.(1966a))
this was not found. However, it was found again with a modification of
Rey's diet.

Agar was used by Moore, Tzanakakis (diets M and N) and Orphanidis,
but its use involves the heating of the medium, which is another source of
variation (Tzanakakis et al.(1966)), and if its use can be avoided, so much the
better. The use of agar therefore seems to have been introduced because
the larvae gnaw their way through a compact medium, the olive mesocarp.
But, after the use of substrates based on bran and corncob, agar proved
not to be necessary.

Protein sources

The protein hydrolysate used by Hagen et al.(1963), after Moore's
casein (1959) and peanut butter, marked an important step, considerably
increasing yield, so it was thenceforth included in all other diets
(Orphanidis et al.(1963), Tzanakakis et al.(1966a), Tzanakakis and
Economopoulos (1967), Pelekassis and Santas (1969)) up to Rey's use
of germinated chick peas (1969). This was an excellent idea, taking
advantage of natural protein hydrolysis during histolysis of seeds, pre-
ceding the histogenesis of seedlings. The costly enzymatic protein hydro-
lysate could then be discarded for a very cheap material.

Meanwhile, various attempts at using partially hydrolysed protein,
or a mixture of crude protein and smaller amounts of hydrolysate (at
Oeiras) were not successful, with the exception of the diet of Orphanidis
et al.(1969). The choice of chick peas was very fortunate since some
other legumes tried out did not work [Rey: lupinus (Lupinus albus),
vetches (Lathyrus sativus), soy beans (Soja hispida); Silva: Lupinus luteus
and L. albus, both bitter and sweet, Lathyrus cycera and Vigna sinensis].
They also failed as sources of crud and hydrolysed protein (e.g. Tzanakakis
et al.(1966)).

Roasted peanuts were introduced by Tzanakakis et al.(1966a) and
Tzanakakis and Economopoulos (1967) with good results. It is not known
whether its active component is the protein, the oil, or something else.

Carbohydrates

Sucrose is generally included in all diets. Only recently the intro-
duction of germinated chick peas has enabled Rey (1969) to drop it. The
only other sugar used was fructose, by Moore (1959).

Mannitol, used by Moore, is still included in the formula of Pelekassis
and Santas (1969) but without conclusive reasons for its use, as far as is
known.

Lipids

Removal of olive oil from the diet considerably reduces the yield
(Lopez D. (1965)). Its active component is not yet known for certain.

With the oil, an emulsifier, mostly Tween 80, is normally used, but has now been discarded by Rey (1969), after the introduction of chick peas. Peanuts also provided some fat.

Salts

Moore (1959) used Salt Mixture No.2 and Hagen et al.(1963) used the Wesson salt mixture, but afterwards the need of oligoelements were supposed to be provided by the yeast. Nevertheless, some tests were done with mineral salts of K, Cu, Zn, Mn, Mg and B, according to the percentage found in the analysis of the olive pulp ash (Silva), but results did not show any improvement. Ashes, as complementary source of salts, were added by Rey and Silva, also without any results when using percentages based on those found in the olive pulp. Rey's suggestion that the bad results with one brand of yeast were due to lack of copper is not borne out by other experiments (Silva), where bad results were obtained using yeast with a considerably higher copper content.

Vitamins

The source of the vitamins, mainly the B complex, is yeast, a constant item in diets. This is one point still waiting to be completely cleared up, and we feel this is not possible without some tests including the basic vitamins.

As a matter of fact, the yeast quality has proved a fundamental point, yields depending very closely on the source of the yeast used. Still, some of the experiments gave rather puzzling results (Silva, Rey, Tzanakakis), a different yeast being shown to be the best in each case. Perhaps this can be explained by the use of different strains of flies, with different grades of "domestication", i.e. with more or less generations completed in the laboratory. The solution can only be found by comparison in the same place with strains of different origin. These conditions have been applied at Oeiras, where Greek and Spanish pupae have been sent, and experiments are being carried out.

Of course, the somewhat erratic activity of the yeasts is to be expected, if one considers the number of active constituents and the different grades of purification applied to the commercial brands. Only purified brands can guarantee a constant composition. But the cost is at least 2.5 times that of the cheaper products. Choline chloride, used by Moore (1959, 1962) and Lopez D. (1965) is still adopted by Orphanidis et al.(1969) but not by other researchers, as satisfactory yields are obtained without it.

Steroids

Cholesterol (and sitosterol used in the first diet of Moore) has not been included in other diets, but it is not clear why.

Preservatives

As the diet for mass rearing will inevitably be used in non-aseptic conditions, it is necessary to use anti-fungal, anti-yeast and anti-

bacterial substances, which should cause the least possible adverse action on the larvae. Owing to the favourable circumstance that larvae can stand a very high acidity of the diet, most bacteria are easily avoided by lowering the pH by adding hydrochloric acid. This practice is universally accepted after Hagen et al.(1963), with pH's of 3.8-4.2 as the values mostly used (Orphanidis et al.(1969), Rey (1969), Tzanakakis and Economopoulos (1967), Silva)}. For mould and yeast inhibition, the two preservatives most frequently used are methyl parahydroxybenzoate (Nipagin, Tegosept) and potassium sorbate (or sorbic acid). However, the toxicity of Nipagin, more acute in acid medium, calls for its replacement or reduction.

The recent use of germinated sprouts by Rey (1969) has allowed its percentage in the diet to be lowered by one half to 100 mg/100 g, with the acceptance of some fungistatic action of the germinating legume. However, the difficulty of standardizing this germination causes sometimes an unprotected batch of diet, mainly when corncob is used (Rey, Silva).

Many other chemicals have been tried, without much success, e.g. antibiotics — Nistatin, cycloheximide, tetracyclin, polymicin, mycostatin; chemical fungicides — metiolate, captan, maneb, zineb and dipholatan (Rey); also sulphaguanidine and 8-quinolinol, quinosol and salicylic acid (Tzanakakis et al.(1966a)). Only Delvocid (5% pimaricin) has given some promising results in a very low concentration, but its level of toxicity has not yet been exactly defined. Sodium benzoate and butyl parahydroxybenzoate (Butoben) have also been used (Lopez D.(1965), Orphanidis et al. (1963), Hagen et al.(1963)).

Density of larvae, thickness of the substrate

As has been stated, the natural condition of solitary life does not favour a heavy concentration of larvae in the substrate and so leads to a low use of its constituents. Densities over 1 larva/g of medium normally cannot be exceeded without loss of efficiency (Tzanakakis et al.(1967a)). To get a better output, Tzanakakis (1969) is now re-using the medium, after some supplementing of soy hydrolysate and brewer's yeast, with promising results.

In addition, selling the used medium as feed for chicken or cattle, as has been done for the C. capitata medium, can also contribute to lowering the costs. The thickness of the medium is normally about 1.5-2.5 cm. Rey (1969) found no difference between 0.6 cm and 1.7 cm. This thickness can be increased, we think, with substrates containing corncob, due to the better aeration it provides, allowing perhaps the use of more convenient containers. The containers used have been of numerous types, but mostly Petri dishes for experiments and shallow pans for more extensive rearing.

Pupae

Either pupae are removed individually from the medium or the larvae are allowed to crawl outside the container to fall down into a tray provided with sawdust where they pupate (Tzanakakis and Economopoulos (1967)) and can then be sifted out. Also, a large variety of containers have been used to hold the pupae until emergence, under dry or slightly moist conditions.

Sexing can be done by observing the setal fringe on the 3rd abdominal tergite of the males during the last days (Tzanakakis et al.(1967b, 1968)). Alcohol dipping, followed by water dipping, facilitates the observation.

Concerning the rearing in general, it seems that the main handicaps are that much more space is needed than for other fruit flies, and that the diet is not yet completely adequate. Progress is nevertheless encouraging, with the use of Parafilm, with still lighter and cheaper cages, and with more automatic recovery of eggs.

The same applies to the diet: after the breakthrough of the use of chick pea sprouts in a medium with ground corncob, it seems that no essential component is now lacking and the improvement should come more probably in the direction of a more balanced composition of their constituents (Friend (1968)), following a standardization of such fundamental ones as yeast and germinated chick peas.

Formula N of Tzanakakis was accepted as a standard one for comparisons with other formulae at the FAO Athens meeting in 1969.

3. RADIATION

The first reported experiments for sterilizing $\underline{D}$. oleae as pupae were performed by Melis and Baccetti (1960) in Italy during 1960. This work was remarkable as it established the range of gamma rays needed to sterilize the males (8000-1200 rad) and the best period for applying the radiation (3-7 days before emergence), and, in recognizing the capability of competition of the sterilized males, found in a field experiment, a 4:1 proportion of sterilized versus normal males to be enough for control (only 1% of eggs laid developed into larvae). These findings were later published separately by Baccetti and Cappellini (1961), giving more details on the radiation technique using a ^{60}Co source. Later, the histolesions produced by the ionizing radiation to the mesenterion were studied by Baccetti et al. (1961).

Thomou (1963), in Greece, reproduced practically all these findings, also using larvae for irradiation which at 2000 ± 300 rad remained sterilized throughout their adult life.

For adults, 15-18 krad was found to sterilize them completely throughout their life. Baccetti and Bairati (1964) describe in very close detail all the structures connected with male gonads and male germ cells. The histolesions caused by gamma radiation were reported by Baccetti (1965). The main findings were:

The sheaths of the testes are not affected.

Late spermatogonia and particularly the first spermatocytes are the most sensitive cells.

Young spermatogonia appear to be rather insensitive.

The evolution of the spermatids is not blocked, but they are sometimes damaged in ultrastructure.

The bundles of spermatids remain constant (256 each).

Single cells in the bundles of spermatids degenerate.

Tsitsipis and Tzanakakis (1968), on the question of male competitiveness, concluded that mating ability was unimpaired at 8 krad. Tzanakakis

et al.(1966b) also confirmed the effectiveness of the 8-12 krad dose, using laboratory-reared pupae; 6 krad was also considered satisfactory. Later it was found (Tzanakakis et al.(1967b)) that with the 8-krad dose, 20 times fewer punctures were made by the sterilized females.

Pelekassis and Manikis (1963) concluded that the ratio of sterilized males must be increased to 8:1 in order to achieve a low (4%) fertility level of eggs laid in caged trees.

Concerning the mating ability and frequency, it was found that sterilized males were capable of mating daily for 30 days, just as normal males, but were able to convey sperm only up to the 5th-11th mating, after which the testes were found to be depleted at dissection (Tzanakakis (1969)).

4. CHEMOSTERILANTS

The use of chemicals for inducing sterility has also been investigated for the olive fly. Interest in chemosterilants has practically arisen only in the last decade. It was during 1963 that the first mention was made of applying these to the olive fly. This was the work in 1962 by Orphanidis and Patsakos (1963). The chemical used was metaphoxide (metepa); at 1% it sterilized only males. Pelekassis and Manikis (1963) found tepa lethal at 1% to both sexes.

A preliminary field experiment, including 2000 olive trees and using apholate, shows some reduction of the egg fecundity from 89.7% to 45.5% (Orphanidis (1965), Orphanidis et al.(1966)).

Tzanakakis et al.(1967b) found differences in the action of metepa (1%) added to the solid sucrose or to the standard liquid diet: it was mostly lethal with the sucrose and produced 100% sterility to females and 90% to males, when added to the diet. Metepa was found to sterilize both males and females at 1%. With tepa (aphoxide) a few dosages were tried:

1% — lethal to both sexes, young and older flies
0.75 - 0.125% — lethal to young flies
0.1% — not lethal to older flies
0.025 - 0.01% — no ovary development, young flies
0.005-0.0025% — sterilized young females
0.0025-0.00125% — sterilized young males.

These doses are much lower than the ones reported by Orphanidis and colleagues. Fytizas (1967a, b, 1969a, b) and Fytizas and Bacoyannis (1967, 1968a, b, 1969a, b) did a considerable amount of work, the conclusions of which have been summarized by Tzanakakis (1969).

The need to evaluate the effects of chemosterilants was emphasized in order to separate the main effects (genetic effects on the spermatozoa and on the eggs, cytotoxic effects on the ovaries and inhibition of oviposition) from the secondary ones (lethal effects on both sexes, cytotoxic effects on the testes and mating inhibition).

Tepa is considered to have the most favourable properties, since the doses inducing the lethal, the cytotoxic and the genetic effects are widely separated, with a very favourable safety factor ($\times 50$).

There seems to be no connection between the action of tepa on the one hand and male mating drive and oviposition mechanism in females on the other; this looks very favourable in practice. The same result was found with reserpine (Fytizas and Bacoyannis (1968a)). In this case, it is the oviposition that is inhibited (at the 0.1% dose) but the eggs are formed normally in the ovaries. At higher doses (0.5, 1.0 and 2.0%) the development of the ovary was inhibited in part. This is a very interesting line of work, offering a different way of reducing the reproductive capacity of the pest.

REFERENCES

BACCETTI, B. (1965). "The action of radiation on the male germ cells", Advances in Insect Population Control by the Sterile-Male Technique (Report Panel, Vienna, 1965) IAEA, Vienna pp. 8-11.

BACCETTI, B., BAIRATI, A. (1964). Indagini comparativi sull' ultrastruttura delle cellule germinali maschii in Dacus oleae ed in Drosophila melanogaster — I. Redia 49, pp. 1-30.

BACCETTI, B., CAPPELLINI, M. (1961). Ricerche sulla sterizzazione di insetti nocivi con radiazioni ionizzanti, Nunt. radiol. 7, pp. 600-3.

BACCETTI, B., de DOMINICIS, RAFFAELLA, CAPPELLINI, M. (1961). Modificazioni istochimichi ed ultrastrutturali indotti dalle radiazioni ionizzanti sul mesentero di Dacus oleae, Redia 46, pp. 205-22.

CAVALLORO, R. (1967). Orientamenti sull' allevamento permanente di Dacus oleae Gmel. in laboratorio, Redia 50, pp. 337-44.

FRIEND, W. (1968). "The nutritional requirements of Diptera", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests (Proc. Panel Tel Aviv, 1966) IAEA, Vienna, pp. 41-57.

FYTIZAS, E. (1967a). Action du Tepa sur les adultes de Dacus oleae Gmel. en fonction des quantités du chimiostérilant utilisé. Annls Inst. phytopath. Benaki 8, pp. 32-46.

FYTIZAS, E. (1967b). Influence de quelques facteurs sur l' action stérilisante du Tepa sur les femelles de Dacus oleae Gmel. Meded Rijksfacult. Landbouwwetensch. Gent 32, pp. 717-25.

FYTIZAS, E. (1969a). Action de la lumière et de la température sur l' efficacité stérilisante du Tepa, Annls Épiphyt. (sous presse).

FYTIZAS, E. (1969b). Effets cytotoxiques du Tepa sur les mâles du Dacus oleae. Meded. Rijksfacult. Landbouwwetensch. Gent 34, pp. 637-42.

FYTIZAS, E., BACOYANNIS, A. (1967). Action du Tepa sur des femelles de Dacus oleae Gmel. sexuellement mures. Meded. Rijksfacult. Landbouwwetensch. Gent 32, pp. 917-25.

FYTIZAS, E., BACOYANNIS, A. (1968a). Action de la réserpine sur l' oviposition de Dacus oleae Gmel., Annls Épiphyt. 19, pp. 623-8.

FYTIZAS, E., BACOYANNIS, A. (1968b). "Action du Tepa sur le développement nymphal de Dacus oleae Gmel.", Isotopes and Radiation in Entomology (Proc. Symp. Vienna, 1968) IAEA, Vienna, pp. 209-15.

FYTIZAS, E., BACOYANNIS, A. (1969a). Action stérilisante du Tepa (temporaire et permanente) sur les mâles de Dacus oleae, Compte rendu III Congr. Intern. des Antiparasitaires.

FYTIZAS, E., BACOYANNIS, A. (1969b). Effets stérilisants et effets secondaires du Tepa sur les adultes de Dacus oleae Gmel., 8th FAO Meeting on the Control of the Olive Pests, Athens, May 8-12, 1969.

HAGEN, K., SANTAS, L., TSECOURAS, A. (1963). "A technique of culturing the olive fly Dacus oleae Gmel. on synthetic media under xenic conditions," Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963) IAEA, Vienna, pp. 333-56.

LOPEZ-D.,F. (1965). "Olive fly (Dacus oleae Gmel.) rearing in Greece," Advances in Insect Population Control by the Sterile-Male Technique (Report Panel, Vienna, 1965) IAEA, Vienna pp. 4-6.

MELIS, A., BACCETTI, B. (1960). Metodi di lotta vecchi e nuovi sperimentati contro i principali fitofagi dell' olivo in Toscana nel 1960. Redia 45, pp. 193-217.

MOORE, I. (1959). A method for artificially culturing the olive fly (Dacus oleae Gmel.) under aseptic conditions, Ktavim 9, pp. 295-296.

MOORE, I. (1962). Further investigations on the artificial breeding of the olive fly Dacus oleae Gmel. under aseptic conditions, Entomophaga 7, pp. 53-57.

MOORE, I., NAVON, D. (1965). "Rearing medium for Dacus oleae Gmel.", Advances in Insect Population Control by the Sterile-Male Technique (Report Panel, Vienna, 1965) IAEA, Vienna, pp. 6-7.

ORPHANIDIS, P. (1965). Rapport sommaire sur les recherches phytopharmaceutiques relatives à la lutte contre le Dacus oleae et les autres ennemis de l' olivier effectuées en Grèce pendant 1963-65, Vième Réunion de la FAO sur la lutte contre la mouche de l'olive, Madrid, 17-24 May, 1965.

ORPHANIDIS, P., PATSAKOS, P. (1963). Recherches en laboratoire sur la stérilisation d' adultes de Dacus oleae Gmel. au moyen de métaphoxide (Comparaison avec les résultats relatifs obtenus sur le Ceratitis capitata Wied.), Annls Inst. phytopath. Benaki 5, pp. 305-22.

ORPHANIDIS, P., SOULTANOPOULOS, C., PATSAKOS, P. (1963). Contribution à l' étude d' une méthode d' élevage du Dacus oleae Gmel. sur substrat artificiel, FAO/IAEA Study Group on Radiation and Isotope Applications in olive fly control, Lisbon, May 24-25, 1963.

ORPHANIDIS, P., PATSAKOS, P., KALMOUCOS, P. (1966). Expérience préliminaire en plein champ sur la chimiostérilisation d' adultes du Dacus oleae Gmel., Annls Inst. phytopath. Benaki 7, pp. 177-90.

ORPHANIDIS, P., PETSIKOU, N., PATSAKOS, P. (1969). Eléments sommaires concernant un élevage expérimental du Dacus de l' olive sur substrat artificiel, 8ème Réunion de la FAO sur la lutte contre les parasites de l' olivier, Athènes, Mai 8-12, 1969.

PELEKASSIS, C., MANIKIS, P. (1963). Preliminary note on the chemical sterilization of the olive fruit fly, 5th FAO Meeting on the Control of Olive Pests, Lisbon, May 20-27, 1963.

PELEKASSIS, C., SANTAS, L. (1969). On a new improved larval artificial medium of Dacus oleae Gmel., 8th FAO Meeting on the Control of Olive Pests and Diseases, Athens, May 8-12, 1969.

REY, J.M. (1969). Development of a larval diet for the rearing of Dacus oleae Gmel., 8th FAO Meeting on the Control of Olive Pests and Diseases, Athens, May 8-12, 1969.

THOMOU, H. (1963). "Sterilization of Dacus oleae by gamma irradiation," Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963) IAEA, Vienna, pp. 413-24.

TSITSIPIS, J., TZANAKAKIS, M. (1968). "Recent radiation work with the olive fly at the "Democritus" Nuclear Research Center, Greece", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests (Proc. Panel Tel Aviv, 1966) IAEA, Vienna, pp. 117-121.

TZANAKAKIS, M. (1969). Experiments with the olive fruit fly at the "Democritus" Nuclear Research Center, Greece, 1967-1968 (A summary of progress), 8th FAO Conference on the Control of Olive Pests and Diseases, Athens, May 8-12, 1969.

TZANAKAKIS, M., ECONOMOPOULOS, A. (1967). Two efficient larval diets for continuous rearing of the olive fruit fly, J. econ. Ent. 60, pp. 660-2.

TZANAKAKIS, M., ECONOMOPOULOS, A., TSITSIPIS, J. (1966a). Improved artificial food media for larvae of Dacus oleae (Gmelin), Z. angew. Ent. 58, pp. 373-83.

TZANAKAKIS, M., TSITSIPIS, J., PAPAGEORGIOU, M., FYTIZAS, E. (1966b). Gamma radiation-induced dominant lethality to the sperm of the olive fruit fly, J. econ. Ent. 59, pp. 214-16.

TZANAKAKIS, M., ECONOMOPOULOS, A., TSITSIPIS, J. (1967a). The importance of conditions during the adult stage in evaluating an artificial food for larvae of Dacus oleae (Gmelin). Z. angew. Ent. 59, pp. 127-30.

TZANAKAKIS, M., TSITSIPIS, J., ECONOMOPOULOS, A., FYTIZAS, E. (1967b). Recent studies with Dacus oleae (Gmelin) at the "Democritus" Nuclear Research Center, Greece, 7th FAO Meeting on the Control of Olive Pests, Palermo, 2-10 May, 1967.

TZANAKAKIS, M., TSITSIPIS, J., STEINER, L. (1967c).   Egg production of olive fruit fly fed solids or liquids containing protein hydrolisate, J. econ. Ent. 60, pp.352-4.

TZANAKAKIS, M., ECONOMOPOULOS, A., TSITSIPIS, J. (1968). "Artificial rearing of the olive fly: progress report", Radiation, Radioisotopes and Rearing Methods in the Control of Insect Pests, (Proc. Panel Tel Aviv, 1966) IAEA, Vienna, pp. 123-30.

REVIEW OF OLIVE-FLY ECOLOGY IN RELATION TO THE STERILE-MALE TECHNIQUE

P.A. MOURIKIS, E. FYTIZAS
Department of Entomology,
Benaki Phytopathological Institute,
Kifissia, Athens, Greece

Abstract

REVIEW OF OLIVE-FLY ECOLOGY IN RELATION TO THE STERILE-MALE TECHNIQUE.

Data on average monthly catches of Dacus oleae (Gmelin) are presented for the years 1966 and 1967 as compared with mean temperature and relative humidity. In both years the population peak was in October and four generations could be shown. Laboratory experiments indicate that adults must be present in olive groves even during the colder part of the year. Results of radiation work by different authors is discussed. The need is mentioned of a powerful attractant in order to determine population levels in olive groves, longevity and field behaviour of released sterile flies, and migrations from one group of orchards to the other ones. Work with chemosterilants, namely tepa, is reviewed.

INTRODUCTION

The olive tree is one of the most important cultivated plants in many Mediterranean countries. In Greece there are about 85 million trees located mainly along the coastal areas and in the islands. The most important pest of the olive fruit is Dacus oleae (Gmelin) and yearly it causes damage estimated at about 20-25% of the crop.

Because of its great economic importance, the biology and the control of this insect has been studied for years, and a program has recently been planned for the application of the sterile-male technique.

ECOLOGY OF Dacus oleae

Apart from the study of Moore (1960), the ecology of D. oleae has not been particularly examined. However, data on different ecological aspects have been published in articles concerning biology and control of Dacus, such as those of Martin (1952), Avidov (1957), Sakantanis (1952 a, b, 1954) and Pelekassis (1960).

In Greece as well as in other olive-oil-producing countries in the Mediterranean area the population peak of D. oleae, as well as highest attack on the fruit, has been noticed in autumn, particularly from the beginning of September until about the middle of November, the date varying with climatic conditions. This has been attributed, apart from other causes, to the migration of the insect from mountainous areas, where there are cultivated and wild olive trees, towards olive orchards located in the plains. Moore (see Avidov (1957), p.114) noticed that the fecundity of the females during autumn was twice as high as in summer. Ayoutantis et al. (1954) attribute the reduction of egg laying during summer to the

TABLE I.   AVERAGE MONTHLY CATCH PER TRAP[a] OF Dacus oleae (Gmelin) ADULTS AND THE CORRESPONDING TEMPERATURE AND RELATIVE HUMIDITY IN SIPIAS — ROVIES (EUBOEA)

Month	1966			1967		
	Monthly catch	Mean temperature (°C)	Mean relative humidity (%)	Monthly catch	Mean temperature (°C)	Mean relative humidity (%)
January	2.2	8.1	80.15	6.5	8.2	73.71
February	8.3	12.6	73.60	4.2	7.3	78.62
March	11.3	11.7	73.35	10.3	10.7	75.95
April	16.3	16.8	70.60	21.2	15.1	72.87
May	2.0	20.1	67.25	7.8	20.5	70.40
June	26.9	24.5	66.25	25.1	24.1	62.46
July	27.2	28.3	60.66	59.4	26.8	59.48
August	40.4	28.2	68.81	106.3	27.6	61.82
September	28.1	22.7	65.62	83.9	23.0	68.46
October	128.4	21.5	71.78	158.7	18.8	76.77
November	82.3	15.5	79.71	96.1	13.6	81.05
December	17.8	11.0	78.70	25.5	10.3	82.51

[a] McPhail traps containing a solution of 3% diammonium phosphate.

TABLE II.   EFFECT OF TEMPERATURE 5 ± 1°C ON THE VARIOUS STAGES OF Dacus oleae (Gmelin) IN THE DARK (ICEBOX)

Stage	Mortality (%)	in days	Remarks
Egg	100	15	in olives
Larva I	100	15	
Larva II	100	15	
Larva III	100	15	
Pupa	100	15-30	
Adult	90	30	

low relative humidity of the season. This observation is echoed by Bateman's (1968) statement that a considerable increase in the fecundity of Dacus tryoni is noticed in years of high moisture.

     In Table I we give data on the trapping of adult D. oleae in the olive orchard of one area (Euboea-Rovies-Sipias) during the years 1966 and 1967, together with the corresponding meteorological data.

In Table II are given data, obtained under laboratory conditions, on the effect of low temperature on different stages of the insect.

From Tables I and II it is clear that adults of the insect are found in the olive orchard and "theoretically"[1] fly during the whole course of the year.

According to Moore's (1960) data on the development of the insect from the egg to the adult as a function of temperature, the constant is 341 day-degrees C (threshold of development 10.7°C), and on the assumption that the populations of Israel and Greece are biologically similar we should estimate the number of generations in most areas in Greece from 4 to 5 during the periods of summer and autumn when the average temperature is above 11°C. The experiments on a number of generations of this insect under field conditions made by Arambourg (personal communication, 1969) have not yet been completed.

A method for determining the time intervals between generations of D. oleae in the field has been applied in olive orchards in Greece by the Department of Entomology of the Benaki Phytopathological Institute for many years. This method is based on the following data:

    (a) Sampling of olive fruits every 5 days to estimate the infestation.
    (b) Number of adults caught in McPhail traps, also every 5 days.
    (c) Maturity of the female adults.
    (d) Sex ratio of the captured adults.

All these data provide some information for predicting the size of the next generation. Consequently the theoretical estimate of the generations on the basis of Moore's data should be compared with those of field studies.

Kalopissis et al. (1954) estimated the increase of the population under favourable conditions, showing that for one female of the first generation (spring), if she lays 200 eggs the number of oviposition punctures in the 3rd generation will be 200 000 000.

This theoretical estimate does not apply in nature, because many factors prevent the increase of the population. Among these factors are:

    (a) The impossibility of the adults of the first generation laying a sufficient number of eggs;
    (b) The mortality at the immature stages of the insect. In summer, in a dry area (Attica) according to Stavraki (1969), it can amount to 30 - 93% depending on the season, with olive table varieties. On the other hand, on the olive oil varieties during September the mortality ranges from 10 - 18%;
    (c) The parasitism by Hymenoptera which, in various parts of Greece, reach 67.5% or more according to Isaakides (1956).

The most important natural factor which causes the reduction of the insect population during the summer period is the high temperature, which, according to Avidov (1957), prevents the female from laying her eggs. But during autumn there is a rapid increase in the population, due to the favourable seasonal conditions which permit fast development of the insect in a relatively short time.

Unless they are controlled, these generations from September until November create populations which destroy the crop.

---

[1] Theoretically because during the winter, with low temperatures, no flight has been noticed although there are adults in the olive trees.

It is known that the Dacus population, as well as the degree of its attack of the olive fruit, is not identical in different olive orchards in the same area. It appears that the greatest attack and the greatest population chiefly occur in certain places as a result of the climatic conditions of the micro-environment, particularly the relative humidity. According to our observations, in olive orchards with identical climatic conditions, the infestation is greater where there is more fruit.

Until now the only satisfactory methods of controlling the insect are (a) cover spray (with organophosphorous insecticides (Martelli (1950)) and (b) bait spray (with protein hydrolysate + organophosphorous insecticide (Orphanidis and Soultanopoulos (1962)).

These methods are applied from the summer onwards. Cover spray is applied on a small scale because of the difficulty in water supply and the problem of the residues in the olive fruit and the olive oil. Bait spray gives sufficient protection to the crops, but it should be applied over large areas to prevent the danger of reinfestation. The adults of Dacus, as already stated, are found in the olive orchard throughout the year. During winter and spring the insect is found mostly as pupae, in the soil. These pupae, with the rise of the temperature during spring up to May, produce adults that start the initial population that will attack the new crop (Pelekassis (1960)). The new crop begins in most olive-producing districts of the Mediterranean from June onwards.

As to the migration of D. oleae, Lupo (1943) considered that the insect makes two seasonal migrations. The first, in June-July towards the mountain regions, and the second, in September-October to the plains. The migration of Dacus has been generally agreed. Pelekassis et al. (1962) noted movement of Dacus during October from a mountain to a plain olive orchard, over a distance of about 4000 m. Orphanidis et al. (1962) recorded a dispersal of Dacus within a distance of 2000 m in January. In the same season (December) of the year, Baldwin et al. (1967) observed a dispersal of Dacus of no more than 150 m, perhaps because of adverse climatic conditions.

STERILE-MALE TECHNIQUE

Knipling (1955) was the first to describe the potentials of the sterile-male technique for the eradication of the population of an insect. The technique has already been successfully applied in the eradication of Callitroga hominivorax.

Steiner et al. (1962) applied the technique in an isolated area in Hawaii against Ceratitis capitata in an orchard containing a variety of deciduous fruits and other hosts. They succeeded in reducing the population by 90% during the period before the end of the release and after it. But later the population greatly increased, probably through migration of the insect from other areas. The eradication of Dacus cucurbitae in the island of Guam was completely successful (Steiner et al. (1965)).

Although experimental data on the efficacy of this method against Dacus oleae, under laboratory conditions or on a small scale in the field, are limited, we should mention the work of Melis and Baccetti (1960) and Manikas and Baldwin (1967). The latter, during the months October-November, covered olive trees with cages and released sterile and normal

adults in ratios of 4:1, 6:1 and 8:1. They noticed that during January the infestation in the fruits of the control trees was about 80% (pupae), whereas in trees where the sterile-to-normal ratio was 8:1 the infestation amounted to 4%. According to them, a ratio of 8:1 and above is sufficient to eradicate D. oleae. However, the above-mentioned workers did not take into consideration the possibility of change in the population due to adult dispersal or migration from neighbouring areas.

Among the assumptions made by Knipling (1955) about the successful application of the sterile-male technique is that the sterile males (irradiated by gamma rays or other methods) should not be lacking in the ability to mate nor in their longevity, as compared with the normal males of the natural population. With D. oleae, Tzanakakis (1967) did not find any increased mortality of sterile males in comparison with normal ones. These sterile males were produced from pupae subjected to gamma rays at doses of 6 - 12 krad. The same author showed that in the laboratory in the case of matings between sterile males and normal females or normal males and normal females, the male was able to mate with at least 2 females during a period of 7 days, even when the proportion was 1 male to 4 females. Fytizas (1969) observed that the male mates almost every day in the course of a month. Tzanakakis and Tsiropoulos (1969, unpublished data) found that several irradiated and normal males could mate normally every day for 30 consecutive days if in the cage where the male was maintained one virgin female was added daily and removed after mating.

As to the female, Zouros and Krimbas (1969) proved that in the natural population it is not polygamous, bigamous matings amounting to only 10%. On the basis of Tzanakakis' data, males emerging from pupae irradiated at a late stage by 8000-rad gamma rays showed a mating ability and competitiveness not inferior to normal males. From these facts it would seem that the method will be efficacious for D. oleae. This assumes that the artificial rearing methods developed by Moore (1959), Hagen et al. (1963), Santas (1965), Tzanakakis and Economopoulos (1967) and Rey (1969) will be applied on a mass-production scale. There is in addition a need for knowledge of the adaptation of the artificially produced population under field conditions.

Steiner et al. (1965) have successfully applied the sterile-male technique against the related species, Dacus cucurbitae.

For the application of the sterile-male technique in olive orchards, we should require additional knowledge of the following ecological aspects:

(a) Means of estimation of the D. oleae population in the olive orchard. In contrast to the other Dacus species there has not been found so far a powerful attractant that would indicate the level of population in the olive orchard. However, McPhail traps baited with protein or ammonium solutions are somewhat attractive to both sexes of D. oleae. These traps are currently used in Greece to give indications about Dacus population in olive orchards. They are suitable for indicating population density and fluctuation.

(b) The behaviour of sterile adults in the olive orchard and their survival during summer and autumn.

(c) The longevity of the released sterile adults, enabling one to decide on the proper time for the population release. In our opinion this should take place during the periods when the normal population is found at low levels (a period up to the beginning of September for most olive orchards in Greece).

(d) Whether the <u>Dacus</u> migration from certain olive orchards into others takes place in bulk or whether the population dispersal happens in various directions.

CHEMOSTERILIZATION

A.   Effect of tepa on adults

According to the amount administered, it has been found that tepa can cause
(a) The death of adults of both sexes
(b) Cytotoxic damage on ovaries and on testes
(c) Genetic effects on oocytes and spermatocytes.
On the other hand, it has no effect on:
(a) Male vigour
(b) Oviposition of mature eggs.

B.   Rate of effect

We have investigated the rate of effect after its absorption into the insect's body, and note the following :
(a) Cytotoxic activity has been observed 24 hours after application.
(b) Genetic effects were observed 2 to 10 hours after application.
(c) It was impossible to determine the exact time required for chemosterilizing the males.

C.   The persistence of chemosterilization

The persistence of sterilization in females depends on the doses of tepa administered, whereas in the males the duration depends also on the age and on the physiological condition. This difference is due to the fact that in <u>D. oleae</u> females the development in each egg tube is independent and continuous while in males the development of spermatocytes is synchronous and gradual.

D.   Oligospermia and aspermia

The number of spermatocytes affected and the degree of damage to each depends on the quantity of tepa administered, as well as on the degree of sensitivity of the spermatocytes at the time of administration. The effects are as follows:
(a) Genetic effects
(b) Oligospermia
(c) Aspermia.
Seeing that the males of <u>Dacus</u> are polygamous (virtually one mating per day during their sexual life), the oligospermia and aspermia are serious disadvantages in the use of chemosterilization (few or no spermatozoa ejaculated into spermatothecae during mating and consequently no competitiveness with the normal spermatozoa introduced in the spermatotheca of the same female from the former mating with the normal male).

However, the seminal fluid lacking spermatozoa can cause unreceptiveness of the female in the following mating. In this case (inhibition of new mating) the seminal fluid cannot be considered useless.

Accumulation of effects on male

Repeated administration of non-sterilization or low-sterilization doses causes high sterility, higher than that induced by the sum of these doses applied once. The accumulated effects depend first on the number of doses and second on the time interval between doses.

E. Safety factor

The safety factor between lethal and sterile doses is satisfactorily high, being × 50.

For the females the cytotoxic effects as well as the genetic effects are considered to constitute sterilization. For the males, only the genetic effects are considered as sterilization. The cytotoxic effects on the testes resulting in oligospermia and aspermia are as harmful for the application of the chemosterilization technique as lethal action. So it is necessary to determine a safety factor between the doses causing cytotoxic effects and those causing genetic effects. The permanent sterilization of the sperm is induced by approximately the same tepa doses as cause the destruction of the testes.

Fytizas and Tzanakakis (1966) have stated that it is possible to reduce the progeny of the natural population by the use of an antibiotic included in bait sprays applied in olive orchards. This statement has been based on their experiments in which by adding 0.25% streptomycin to the adult diet for only one day, they prevented the development of the larvae from eggs deposited in olive fruit for a period of 18 days. The streptomycin was given from the 4th day onwards.

To sum up: relatively little is known on the ecology of this insect under varying ecological conditions, and this aspect should be studied more profoundly in all olive-oil-producing countries of the Mediterranean area. However, on the basis of current knowledge, especially on the behaviour of sterile adults (produced either by irradiation or chemosterilization), the sterile-male technique is worthy of consideration for the possible eradication or elimination of D. oleae from isolated areas.

REFERENCES

AVIDOV, Z. (1957). Phenology of the olive fruit fly (Dacus oleae Gmel.) in the coastal plain of Israel, J. agric. Res. Sta. Rehovot, Ktavim 8, pp.105-116.

AYOUTANTIS, A.J., PELEKASSIS, C.E.D., ARGYRIOU, L.C., MOURIKIS, P.A., TSAKAS, L.E. (1964). Rapport sur les travaux expérimentaux de la lutte contre le Dacus à l'aide de Parathion à Rovies (Eubée) pendant l'année 1953, Annls Inst. phytopath. Benaki 8 (1), pp. 1-75.

BALDWIN, W.F., MANIKAS, G.P., ZARCADA, P. (1967). Studies on the use of radioactive isotopes in following the movement of Dacus oleae (Gmel.) (Diptera: Trypetidae), Laboratory of the Phytopathology Division of the Ministry of Agriculture, College of Agriculture, Athens, Greece.

BATEMAN, M.A. (1968). Determinants of abundance in a population of the Queensland fruit fly. In: Insect Abundance (SOUTHWOOD, T.R.E., ed.) published for the Royal Entomological Section. Oxford, Blackwell Scientific Publications, pp.119-131.

FYTIZAS, E. (1969). Effets cytotoxiques du Tepa sur les mâles de Dacus oleae. Meded. Rijksfacult. Landbouwwetensch. Gent (sous presse).

FYTIZAS, E., TZANAKAKIS, M.E. (1966). Sur la possibilité d'une lutte contre le Dacus oleae par l'utilisation des antibiotiques, Meded. Rijksfacult. Landbouwwetensch. Gent, 31, pp.782-789.

HAGEN, K.S., SANTAS, L., TSEKOURAS, A. (1963). A technique of culturing the olive fly Dacus oleae (Gmel.) on synthetic media under xenic conditions. In: Radiation and Radioisotopes Applied to Insects of Agricultural Importance (Proc. Symp. Athens, 1963),IAEA, Vienna, pp.333-356.

ISAAKIDES, C.A. (1956). La lutte contre la mouche des olives. Les parasites du Dacus. Comptes rendus de l'Académie d'Athènes 31, pp.195-206.

KALOPISSIS, J.Th., KARAMANOS, G., ORPHANIDES, P., VRETTAKOS, L., MOROS, H., PAPOUTSIS, E. (1954). L'expérience de la lutte contre le Dacus à Kirra (Itéa) 1953 Athènes (in Greek).

KNIPLING, E.F. (1955). Possibilities of insect control or eradication through the use of sexually sterilized males. J. econ. Ent. 48, pp.459-462.

LUPO, V. (1943). L'andamento climatico, la mosca delle olive e sua migrazione, Boll. Lab. Zool. Portici 32, pp.137-177.

MANIKAS, G.P., BALDWIN, W.P. (1967). Large scale tests of the ratios of sterile/normal flies required for the control of Dacus oleae (Gmel.) (Diptera: Trypetidae). Laboratory of the Phytopathological Division of the Ministry of Agriculture. College of Agriculture, Athens, Greece.

MARTELLI, G.M. (1950). Prime prove con gli esteri fosforici contro la mosca delle olive (Dacus oleae Gmel.), Agricolt. Pugliese, Bari, III, 7-8-9.

MARTIN, H. (1952). Contribution à l'étude de la mouche de l'olive Dacus oleae Rossi (Diptera: Trypetidae) en Algérie et en Provence, Mitt. schweiz. ent. Ges. 25, pp.341-348.

MELIS, A., BACCETTI, B. (1960). Metodi di lotta vecchi e nuovi sperimentali contro i principali fitofagi dell'olivo in Toscana nel 1960, Redia 45, pp.193-217.

MOORE, I. (1959). A method for artificially culturing the olive fly (Dacus oleae (Gmel.)) under aseptic conditions. J. agric. Res. Sta. Rehovot, Ktavim 9, 295-296.

MOORE, I. (1960). A contribution to the ecology of the olive fly Dacus oleae (Gmel.), Israel Min. Agric. Res. Sta., Special Bull. 26, pp.35+5.

ORPHANIDIS, P.S., SOULTANOPOULOS, C.D. (1962). Olive fruit fly control with proteinaceous bait sprays. (Comparative results to those obtained with arsenated molasse baits), Annls Inst. phytopath. Benaki, Nouvelle Série 4, pp.103-111.

ORPHANIDIS, P.S., SOULTANOPOULOS, C.D., KARANDINOS, M.G. (1962). Preliminary experiments with radioactive phosphorus (^{32}P on the dispersion of olive fruit fly adults (Dacus oleae (Gmel.)), Annls Inst. phytopath. Benaki, Nouvelle Série 4, pp.155-182.

PELEKASSIS, C.E.D. (1960). Experiments for olive fruit fly (Dacus oleae (Gmel.)) control with organic phosphorous compounds, conducted in Greece, 1958-1959, Paper presented to the 4th FAO Meeting on the Olive Pests Control held in Tel-Aviv, Israel, Dec. 1960.

PELEKASSIS, C.E.D., MOURIKIS, P.A., BANTZIOS, D.N. (1962). Preliminary studies on the field movement of the olive fruit fly (Dacus oleae (Gmel.)) by labelling a natural population with radioactive phosphorus (^{32}P), Annls Inst. phytopath. Benaki, Nouvelle Série 4, pp.170-179.

REY, J.M. (1969). Development of a larval diet for the rearing of Dacus oleae (Gmel.). Paper presented to the 8th FAO ad hoc Conference on the Control of Olive Pests and Diseases, held in Athens, 8-12 May 1969.

SAKANTANIS, K.B. (1952a). Facteurs déterminant le comportement de Dacus oleae (Gmel.), vis-à-vis des variétés d'olives, Revue Path. vég. Ent. agric. Fr. 32, pp.50-57.

SAKANTANIS, K.B. (1952b). Méthode d'élevage au Laboratoire de la Mouche des olives (Dacus oleae Gmel.). Revue Path. vég. Ent. agric. Fr. 32, pp.247-257.

SAKANTANIS, K.B. (1954). Trois ans d'expérimentation contre la mouche des olives (Dacus oleae Gmel.). Geoponika 1, pp.293-299 (in Greek).

SANTAS, L. (1965). On a new medium for the culture of larvae of Dacus oleae. Bull. agric. Bank of Greece No.114 (in Greek).

STEINER, L.F., HARRIS, E.J., MITCHELL, W.C., FUJIMOTO, M.S., CHRISTENSON, L.D. (1965). Melon fly eradication by overflooding with sterile flies. J. econ. Ent. 58, pp.519-522.

STEINER, L.F., MITCHELL, W.C., BAUMHOVER, A.H. (1962). Progress of fruit fly control by irradiation sterilisation in Hawaii and the Mariana Islands. Int. J. appl. Radiat. Isotopes 13, pp.427-434.

STAVRAKI, H. (1969). Mortalité de Dacus oleae (Gmel.) au cours de son développement dans la région d'Attique en 1968. Entomophaga (sous presse).

TZANAKAKIS, M.E. (1967). Control of the olive fruit fly Dacus oleae (Gmel.) with radiation or chemical sterilization procedures. "Democritus" Nuclear Research Center, Athens, Greece. pp.81.

TZANAKAKIS, M.E., ECONOMOPOULOS, A.P. (1967). Two efficient larval diets for continuous rearing of the olive fruit fly, Dacus oleae (Gmel.) (Diptera: Tephritidae). J. econ. Ent. 60, pp.660-663.

TZANAKAKIS, M.E. (1969). Experiments with the olive fruit fly at the "Democritus" Nuclear Research Center, Greece. Paper distributed at the 8th F.A.O. ad hoc Conference on the Control of Olive Pests and Diseases, held in Athens, 8-12 May 1969.

ZOUROS, E., KRIMBAS, C.B. (1969). A study of the number of fertile matings of the female of Dacus oleae (Gmel.) in nature by using enzyme polymorphisms (in press).

FRUIT FLIES OTHER THAN
MEDITERRANEAN FRUIT FLY:
SHORT CONTRIBUTIONS

Summaries of work done
at various institutions

NOTE ON WORK ON THE OLIVE FLY AT ISPRA
R. Cavalloro
Euratom Joint Nuclear Research Center,
Ispra, Italy

Recent research on the autocidal control of fruit flies conducted in
Ispra has included work on the olive fly, Dacus oleae (Gmelin).
Emphasis has been placed on the development of economical mass-
rearing techniques.

Two lines of work have been pursued recently: the development of
a new type of cage for adults, made entirely of screen material (it was
observed that the females freely drop their eggs without the stimulus
of a solid substrate) and the preparation of artificial diets for the
larvae, with ground cornstalks as basic ingredient.

The cages, 20 cm long, 20 cm wide and 25 cm high, are made of
nylon screen (less than 1 mm square mesh). Each one houses 100 couples
of Dacus, which gives them enough freedom of movement, an important
point for this species, and improves survival as a result of better
aeration and more diffuse illumination. After 2 weeks the males are
removed and replaced by the same number of fecund females. The
insects remain in the cage until egg production decreases considerably,
i.e. another 6 weeks.

A solid diet has been developed for the adults, consisting of 4 parts
sucrose, 2 parts 'de-bittered' beer yeast and 1 part enzymatic hydro-
lysate of beer yeast. This mixture is not subject to decay and it is
readily eaten by the insects. It gives good results as shown by the data
and curves on egg production.

This rather spongy diet can be given through the wall of the cage.
Water is supplied similarly by means of a filter paper wetted by a
cotton wick placed in a small water bottle found outside the cage.

The eggs are collected by filtering the water into which they fall,
then placed on a thin layer of diet at the rate of 4000 eggs per kg
and covered by the same.

The search for the best larval diet continues, but promising re-
sults have already been obtained. So far the best low-cost formula is
composed of ground cornstalks to which various nutrients, emulsifiers
and preservatives have been added. On a dry weight basis, this diet
contains 25-30% carbohydrates, 20-25% proteins and 10-15% lipids. Its
pH ranges from 4.3 to 4.5.

The adults placed in the cages described earlier are obtained from
pupae selected for homogeneous size. These are placed in single layers
in opaque boxes containing a small amount of sawdust or sand to offer
the insect a stable substrate at the time of emergence and shortly
thereafter. These boxes are provided with a small circular opening
leading to a transparent test tube where the adults congregate as they
emerge from the pupae.

The right conditions of temperature, relative humidity, photo-
period and asepsy must be maintained for the adults.

Work on Dacus is concerned also with such matters as: fertility
of the successive generations reared on artificial diets, in relation to

the presence of colloidal substances covering the egg at the time of ovideposition; the role of extracellular symbiotic bacteria; the effect of atmospheric pressure.

LATEST INVESTIGATIONS ON THE OLIVE FLY

J. Rey
Instituto Español de Entomología,
Madrid, Spain

Physiology

A common characteristic of all the media so far developed for the rearing of Dacus oleae (Gmelin) larvae is their toxicity for the eggs before the embryonic development. The causes of the mortality could be either the toxicity of some chemicals used in the composition of the diets, or their physico-chemical properties. Among the latter properties, we have started with the study of the effect of the osmotic pressure. Parallel assays of several concentrations of NaCl (a totally dissociated electrolyte) and glucose (a non-electrolyte) were carried out.

As Fig.1 shows, NaCl at a concentration of 4%, equivalent to an osmotic pressure of 31.75 atm, gave a 100% egg mortality, while glucose even at a concentration of 56%, equivalent to an osmotic pressure of 75.96 atm, gave a hatching of 74.28%, similar to that in the distilled water control, which was 76.85%. This difference is not significant at the 0.95 probability level. The results show that the osmotic pressure by itself does not affect egg development. However, the eggs are affected by the ions of the salt used, whether it is the cation Na^+ or the anion Cl^- (Muñiz).

Rearing

Adult feeding and drinking

The Hagen et al. (1963) diet for adults has been slightly modified, 20% of the water being substituted by glycerine. The adult food is thus kept in a fluid state for a period of three weeks, at 60% r.h. This mixture is put on the wire screen of the cages in the form of droplets applied with a special dispenser (Tzanakakis), which saves time in the maintenance of the flies.

We tried to supply the water from outside by means of a cotton wick, but it very often dries up and the device does not seem very promising. At the moment we are experimenting with the wick inside a polyethylene tubing.

Egging

The Hagen et al. (1963) paraffin domes have been replaced by a new device, as a result of some of Prokopy's findings. The cover of a disposable plastic 9-cm.-dia. Petri dish is roughened and 7 holes of 15 mm dia. are made in it. Small domes of a very soft and low-melting-point ceresin are fixed and sealed on these holes. The small
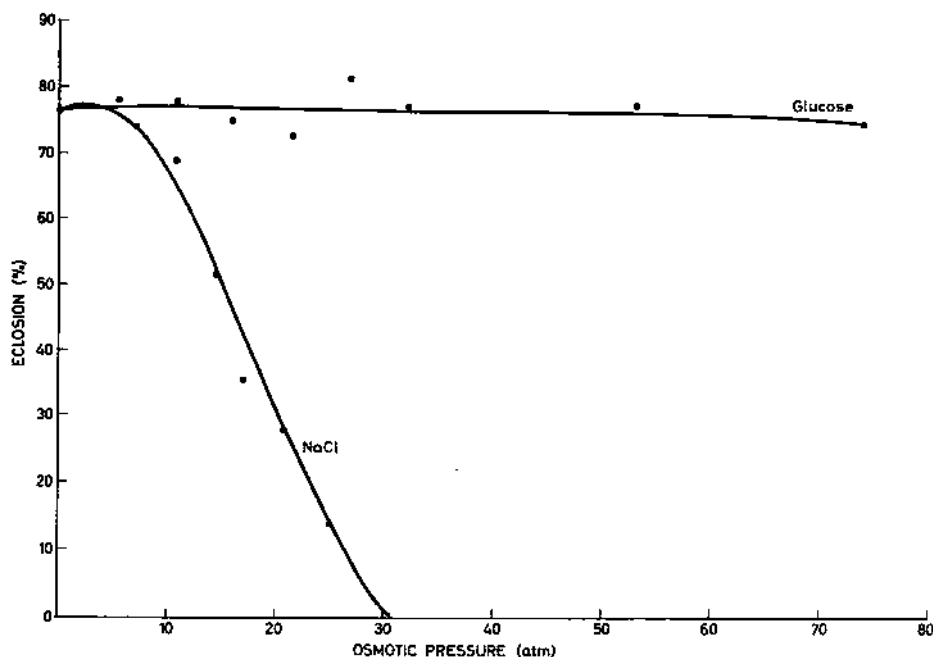
FIG. 1.   Effect of osmotic pressure from NaCl and glucose on egg hatch.

domes are easily made in the usual way by plunging an assay tube of
14 mm dia. into a light soap solution and then dipping them first into
the hot ceresin and finally into cold water. The ceresin is dyed with
a black liposoluble tint. The bottom of the Petri dish is filled with
water to a depth of 2 or 3 mm.

Although we have not quantitative data as yet, observations show
that the flies will lay more eggs than with the bigger, white domes.
The device can be used twenty or more times, but it takes longer to
prepare; altogether it constitutes a saving of time in the handling of the
eggs.

Rearing of larvae

Under a research contract with the IAEA a larval diet was developed
for the artificial rearing of the olive fly. The constitution of the diet
is shown under Diet 1-A-V-18 in Table I.

The total price of 1 kg is 9.45 pts (0.14 US $). With a production of
about 450 pupae per kg, the cost per pupa will be 0.02 pts or 285 US $
a million; this figure includes only the cost of the ingredients. The
size of flies and yield of pupae are similar to those from more ex-
pensive diets. Although the diet represents a very important improvement
over others reported to date, it is still too expensive when one considers
its relatively poor production.

Lately the diet has been changed (see under Diet 1-S-V-3 in Table I)
by reducing the amount of brewer's yeast to 7.5% and substituting the

TABLE I. DIETS DEVELOPED FOR THE REARING OF Dacus oleae (Gmelin) LARVAE

Ingredients	Amounts		Price per unit (1$ = 70 pts)	Cost	
	Diet 1-A-V-18	Diet 1-S-V-3		Diet 1-A-V-18	Diet 1-S-V-3
Distilled water	515 cm³	475 cm³	1.00 pts/litre	0.51 pts	0.48 pts
Eucalyptus paper pulp	115 g	-	14.00 pts/kg	1.61 "	-
Corn cob powder	-	175 cm³	1.00 pts/kg	-	0.18 "
Brewer's yeast (Barcia)	100 g	75 g	25.00 pts/kg	2.50 "	1.88 "
Chickpea sprouts	200 g	200 g	7.00 pts/kg	1.40 "	1.40 "
Olive oil	20 cm³	20 cm³	40.00 pts/litre	0.80 "	0.80 "
Sorbate K (Pfizer)	0.5 g	0.5 g	1.00 pts/g	0.50 "	0.50 "
Nipagin	1.0 g	1.0 g	1.50 pts/g	1.50 "	1.50 "
2N HCl (pH 3.8)	50 cm³	55 cm³	42.00 pts/kg/kg HCl	0.68 "	0.70 "
				9.45 pts (0.14 US $)	7.44 pts (0.11 US $)

eucalyptus paper pulp by corn cob powder (Cavalloro). This powder
gives a better structure to the medium and it takes on a 'bulkier' aspect.
The yields obtained with this diet were 62% ± 10.3 (mean of 21 replicates)
versus 55.2% ± 9.7 (mean of 7 replicates) in Diet 1-A-V-18. In both
cases 80 neonate larvae were sown in 100 g of medium.

The price of 1 kg of the revised diet is 7.44 pts (0.11 US $). With
a production of about 500 pupae per kg, the cost per pupa will be 0.015 pts
or 215 US $ a million. So far this diet has the disadvantage that there is
a higher chance of infestation by moulds.

Any attempt to eliminate the olive oil in both diets has given poorer
yields: 40.1% in the first case and 37.9% in the second. The substitution
of Barcia's yeast by Toruiopsis utilis, a yeast grown on 'alpechin'
with a price one half that of Barcia's, is giving promising results (Rey).

Ecology

The possible overwintering places of the olive fly have been studied
in Spanish areas with a Mediterranean continental climate. The investiga-
tions were carried out at Arganda, near Madrid, in an olive grove whose
fruits were not harvested. During the winter of 1968 and spring of 1969,
olive fruits were picked up at intervals of about 15 days. Some of them
were dissected to assess the percentage of larvae and pupae and others
were left in covered trays to see the number of pupae emerging from
the fruits. At the end of March, traps with ammonium phosphate were
placed in the grove to estimate the presence of adults. The results show
that Dacus oleae can overwinter in the central plateau of the Peninsula,
mainly at the pupal stage (Templado and Murillo).

CURRENT CHERRY FRUIT FLY RESEARCH
E.F. Boller
Fruit Fly Laboratory,
Swiss Federal Research Station for
Arboriculture, Viticulture and Horticulture,
Wädenswil, Switzerland

1. General remarks

In 1962 our research station started its third period of research on
Rhagoletis cerasi L. The first period (1930 - 1944; Dr. R. Wiesmann)
covered general biology and behaviour of the pest and the second (1949 -
1958; Dr. W. Vogel) the application of chemical control methods. The
emphasis of the present research effort is put on ecological studies
(life tables) in connection with the application of autocidal and integrated
control methods.

The strategy of the future control of the cherry fruit fly was
formulated in 1968 and published in 1969. It was decided to carry out
the necessary research for the application of the sterile-insect technique
in suitable regions where mainly table-cherries and cherries for the

canneries are produced (low economic threshold of the pest population) and to search for integrated control methods in areas producing cherries for distillation (moderate infestations to be tolerated).

Research in connection with the application of the sterile-insect technique (rearing, chemosterilization, trapping systems and dispersion studies) was only started in 1966, whereas the integrated control has been the objective of our ecological studies since 1962.

2. Laboratory investigations

2.1. Rearing methods for the cherry fruit fly

2.1.1. Egg production. The lack of a suitable artificial oviposition device was the main obstacle to any successful rearing attempts up to 1965. Now we have developed a suitable method for the mass production of eggs (hollow hemispheres made of a special wax which allow an average production of about 280 eggs per female with a hatch-rate of 91%). The design of an automatic egg-collecting device is in progress.

2.1.2. Larval diets. Some 120 different diets have been tested. Two of them allowed the development of pupae. The main problem to be solved is the physical condition of the diet (texture, humidity). The use of Gelgard (a water thickener; Dow Chemical Corp.) looks promising.

2.2. Sterilization

Preliminary tests with the chemosterilants tepa, apholate and hempa have been carried out with the North-American species R. cingulata and have to be repeated with R. cerasi. Experiments with radiation will be carried out in due course.

2.3. Olfaction

A screening program for potential attractants (food and sex attractants) was initiated early in 1969. The tests are done in a new type of olfactometer developed in 1968 (co-operating agencies: USDA Beltsville; B.C. Research Council, Canada).

2.4. Serological studies

Suitable techniques for the application of precipitin tests (recognition of predators of fruit fly pupae) are studied in the context of an IBP project about pupal mortality factors of fruit flies. Similar studies will deal with the application of radiotracers and radiographic methods.

2.5. Physiological and behavioural studies

The mechanism of diapause initiation must be known and methods for preventing pupal diapause have to be found in the near future in order to establish a continuous rearing program. Behavioural studies in connection with the quality control (sterilized vs. normal flies), the improvement of the general rearing conditions (light intensities and

qualities, oviposition behaviour, etc.) and a future release of sterile flies (colour preferences for trap design, host finding, etc.) have been carried out or are still in progress.

3. Field investigations

3.1. Population dynamics of the pest

Preliminary life tables showed the importance of predators and the meteorological conditions during the oviposition period for the population dynamics of the pest. This long-term study will be continued. The qualitative study is in the process of being changed into a quantitative investigation in order to subject the field data to statistical analysis. We hope that Canadian computer programs can be modified and applied for our problems. Of special interest is the effect of the manipulation of the pupal parasite Phygadeuon wiesmanni on the population dynamics of the pest.

3.2. Dispersion studies

A release of some 4000 marked flies in an experimental cherry orchard (25 ha, ca. 900 cherry trees) was performed in June 1969 in order to study the flight range, the period of maximum flight and the type of plants preferred by the flies in relation to their physiological state. Although the data have not been analysed yet there are strong indications that the cherry fruit fly rarely migrates more than 400 m and does usually stay within 200 m from the place of origin. In addition we could catch the flies in about equal numbers on apple trees, hedges and cherry trees during the pre-oviposition period. This experiment was performed with flies marked with fluorescent powder and some 350 newly designed traps (yellow sticky boards) placed on concentric circles with radii of 50, 100, 200, 300, 400, 500 and 600 m. The largest distance covered by three flies was 500 m.

3.3. Testing new trap systems

The shape, colour and size of new traps were tested in 1969 along with three different sticky materials. Although yellow spheres caught by far the most flies we adopted a yellow rectangle (15 cm × 20 cm) coated with Bird tangle-foot as standard trap that was 200 times more efficient than the normal McPhail-trap with 4% ammonium-carbonate. Further research will be carried out concerning the combination of this visual trap with new attractants and applying the sticky material on disposable plastic foils.

The unexpected efficiency of these traps raised the question whether they could be used as a direct control method. We plan field experiments of this kind next year.

4. Co-operation with international organizations

This laboratory is co-ordinating an IBP project concerning pupal mortality factors of fruit flies of economic importance and participates

in another IBP project concerning olfactory investigations. In addition, it is one of the three European laboratories which are in the process of establishing a working group on the application of autocidal methods against the cherry fruit fly in the framework of the O.I.L.B. (Organisation internationale de lutte biologique).

RESEARCH ON THE CARIBBEAN FRUIT FLY AT THE UNIVERSITY OF FLORIDA

W.G. Eden
Department of Entomology and Nematology,
University of Florida, United States of America

Although Anastrepha suspensa (Loew) has been found, primarily as adults in the regular fruit fly surveillance program in Florida from time to time since 1931, it was in 1965 that it appeared in large numbers and began to cause concern among fruit growers. Since 1965 considerable resources have been devoted to investigations in Florida on A. suspensa (caribfly). A great deal of the work has been co-operatively done by: the Entomology Research Division, U.S.D.A.; Plant Pest Control Division, U.S.D.A.; Division of Plant Industry, Florida Department of Agriculture; entomologists of the University of Florida; and industry.

Hosts

The caribfly is known to attack over 50 kinds of fruit that are grown in Florida. The preferred hosts are guava, Surinam cherry, peach, rose apple, and tropical almond. Other important ones are grapefruit, bell pepper, tomato, avocada, and mango.

Biology

Although mature and overripe fruit is usually attacked, flies will deposit eggs in green fruit. Eggs are deposited singly into the flesh of fruit, usually just under the surface. The eggs hatch in 2-3 days and the larvae feed in the fleshy part of the fruit for 8-9 days. The fruit usually falls to the ground by the time the larvae have matured. Larvae emerge from the fruit and pupate under the soil surface. Adults emerge 12-15 days later. Females begin to lay eggs after 7 days.

Control with Insecticides

Aerial applications of malathion, with and without baits, have been conducted in Florida. Malathion is highly effective in reducing adult populations, and if weekly applications are continued throughout the time required for a life cycle, the caribfly can be very effectively suppressed. In small, single-tree experiments we have had promising results in heavily infested areas with malathion, Sevin, and Dylox.

Mass Rearing

    A culture of caribflies at our Branch Station near Miami is now
in its 13th or 14th generation. Larvae were originally reared in a de-
hydrated carrot medium frequently used for other fruit flies. We are
at present successfully using a dehydrated sugar cane bagasse-citrus
pulp medium that is as good as the carrot medium and is considerably
cheaper. Additional studies on nutrition and physiology are underway.

RECOMMENDATIONS

GENERAL RECOMMENDATIONS

It is the opinion of the panel that fruit flies remain a major, if not the greatest, pest of cultivated fruits throughout the world. Within the past 18 months the technical feasibility of the sterile-insect release method for the control of fruit flies has been proven on a modest scale in several different locations in the world (Nicaragua, Italy and Spain: the Mediterranean fruit fly, Ceratitis capitata (Wiedemann): Guam: the oriental fruit fly, Dacus dorsalis Hendel; Mexico: the Mexican fruit fly, Anastrepha ludens (Loew)). The success in all these experiments suggests that control or eradication of fruit flies by this method is feasible on a much larger scale. However, successful large-scale experiments (1000 km^2 or 400 square miles) are essential before the method can be applied on a practical basis.

At the present time major emphasis is being placed on Mediterranean fruit fly research by national and international organizations. The largest international investment[1] in research and development (facilities and experience) on this fly is in Central America. The group recommends that timely advantage should be taken of these factors and that a large eradication experiment along the lines proposed in the general plan shown in Annex A should be initiated as soon as possible. Apart from Nicaragua, possible sites for such an experiment include Peru, Tunisia and Spain.

International research on the application of the sterile-insect release method for fruit fly eradication or control should be intensified. The objectives of this research should be to develop the necessary methodology and to solve operational difficulties in order that the method will be available to all groups interested in combating fruit flies.

After the successful completion of large-scale experiments (1000 km^2) on selected species, national and regional organizations should be encouraged to eradicate or suppress these species in their areas, using the sterile-insect release method. International support should be made available.

The previous tests of the method involved overflooding isolated or semi-isolated populations with sterile flies. It is important that the feasibility of the method be demonstrated for a progressive campaign, requiring releases over serial portions of the entire infested area. Also, the feasibility of suppressing a portion of an extensive population to obtain economic control needs to be evaluated, integrating the sterile-insect release method with chemical, biological, or cultural control.

Accurate methods of measuring population size and density and the knowledge of biotic potential of fruit flies are needed to provide logistic estimates. The program proposed in Annex A will not provide for the development of these methods but if they are developed elsewhere, they should be incorporated in large programs.

---

[1] OIRSA – Organismo Internacional Regional de Sanidad Agropecuaria
      (Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama)
IAEA – International Atomic Energy Agency
FAO – Food and Agriculture Organization of the United Nations
UNDP – United Nations Development Programme

The panel wishes to emphasize the need for studies of the basic
biology, physiology and ecology of fruit flies and that such information is
vital to successful application of the sterile-insect release method. It
is recommended that such research be supported by national, regional
and international groups.

National and regional organizations should be encouraged to adopt
the methods and approaches developed by international organizations,
thereby allowing the international groups to shift emphasis to insect pests
where the techniques are less developed.

The panel recommends that one or more International Fruit Fly
Research and Training Centres (preferably on a regional basis) be estab-
lished. The centres would conduct research on the application of the
sterile-insect technique for eradication or control of fruit flies of major
economic importance and provide continuing training facilities for qualified
workers interested in adopting this technique. Further, the panel wishes
to emphasize that fruit fly ecology should be stressed at these centres.
In addition, the centres should serve to co-ordinate and assemble informa-
tion on fruit fly distribution and estimates of damage (potential and actual),
and to determine the feasibility and methodology of the sterile-insect
release method for control or eradication on a regional basis. The group
recommends that financing organizations, such as UNDP, World Bank,
etc. be approached concerning the establishment of these centres.

The panel notes the progress achieved through the FAO/IAEA Research
Coordination Program on Fruit Flies and recommends the expansion of
this program as funds become available. The exchange of information
and ideas, and the co-ordination of national and international research
will be of great value in developing the sterile-insect release method
for fruit fly eradication or control.

The panel notes the importance of effective quarantine as a necessary
factor in a fruit fly eradication program and recommends that effective
quarantine procedures be established and strictly enforced in any such
program.

The panel took note of the excellent publications of the FAO/IAEA
relating to the sterile-insect release method. However, it also observed
that these publications were unknown to many entomologists and recom-
mended that FAO and IAEA seek ways of making these publications more
widely known, e.g. by suitable announcements in the FAO Plant Protection
Bulletin and other scientific journals.

SPECIFIC RECOMMENDATIONS

Mediterranean fruit fly, Ceratitis capitata (Wiedemann)

Suppression of Mediterranean fruit fly population using the sterile-
insect release method has been successfully demonstrated in three
independent experiments, carried out in Central America, Italy and
Spain during the last 18 months. These demonstrations involved small,
semi-isolated areas lacking effective plant quarantine services. Apart
from these experiments, additional work has been, and is being, carried
out in other countries with encouraging results.

There are no serious impediments to the operation of a major eradication campaign for this insect. However, in the interest of increasing the efficiency and optimizing the chance for success of this technique it would be desirable to have additional information on the points listed in Annex B.

It is recommended that:

(a) Research work on the subjects mentioned in Annex B should be encouraged and supported by national, regional and international organizations.

(b) It is further recommended that the FAO/IAEA Joint Division and other international bodies give priority to paragraphs 1 through 5 in Annex B, whereas the other paragraphs could be more appropriately dealt with by national and/or regional organizations.

(c) Close co-ordination should exist between all organizations engaged in investigation of the priorities stipulated.

Olive fly, Dacus oleae (Gmelin)

Considerable data are available at present; however, more are required before implementing large-scale control and/or eradication programs. Thorough laboratory evaluation followed by small-scale field tests (of several square kilometres) of the sterile-insect release method should be conducted immediately to determine the areas of research that require further investigation. However, on the basis of present knowledge it is recommended that much of the information listed in Annex B should be derived as soon as possible, especially the diet improvement and nutritional studies and the chemical and physical attractants.

The Anastrepha species

Among the genus Anastrepha, perhaps the most important species causing economic damage are ludens, fraterculus, suspensa, mombimpraeoptans, striata and serpentina. Of this group only A. ludens (Loew) (Mexican fruit fly) has been investigated sufficiently to permit consideration of the sterile-insect release method.

At present sterile Mexican fruit flies are being used to control wild fly populations at the Mexico-California border in Tijuana, Mexico. Recently sterile flies have been released in La Paz, Baja California, in an attempt to eradicate an incipient fruit fly population. However, because of the apparently transitory nature of wild fly populations in these areas, the effect of sterile flies cannot be accurately measured. Also, it is not known if environmental conditions would permit a self-sustaining wild population to exist.

Because of these undetermined factors, it is recommended that an eradication experiment be conducted in an isolated area of about 1000 km^2 (400 square miles) with an established wild fly population, to give a positive demonstration of the sterile-insect release method with A. ludens. In such a test more efficient mass-rearing methods and fly-release techniques could be developed. If successful, this test could perhaps lead to the eventual elimination of this insect pest from North-eastern

Mexico. This could be of economic benefit to Mexican fruit production because costly fumigation of export fruit would no longer be required and the quality and quantity of fruits would be improved.

It is believed that investigations with A. fraterculus in mass-rearing and sterilization techniques are reaching the point at which limited field experiments can be initiated, taking advantage of the completely isolated valleys of Peru.

A. suspensa is now under investigation in the USA. It is hoped that it will be eradicated by the sterile-insect release method. It is therefore recommended that other countries where this species exists initiate basic studies in ecology and biology.

No basic studies with regard to the sterile-insect release method have been carried out with A. mombimpraeoptans, A. serpentina, and A. striata. These should be undertaken once the method has been tested on a large scale for the more important species.

It is recommended that, once the feasibility of eradication of any Anastrepha spp. is proven, more information should be obtained on aspects as outlined in Annex B.

Dacus species, D. dorsalis, D. cucurbitae and D. tryoni

With all three species, methods of rearing and trapping, irradiation-sterility data and chemical control measures are available. The feasibility of population suppression by the sterile-insect release method has been demonstrated.

With respect to D. dorsalis and D. cucurbitae in the western Pacific region and in South-East Asia, the data mentioned in Annex B are urgently needed. The status of current control practices should also be reviewed in the areas where these species are important.

Therefore it is recommended that:

(a) The data for D. dorsalis and D. cucurbitae mentioned in Annex B be accumulated.

(b) On the basis of this information, research relevant to the sterile-insect release method for control of D. dorsalis and D. cucurbitae should be encouraged both to provide information and to generate interest in this field.

(c) Contact with workers on D. tryoni should be maintained and, if necessary, strengthened.

The European Cherry Fruit Fly, Rhagoletis cerasi L.

Background

(1) The European cherry fruit fly is of great economic importance because the species is widespread in Central Europe, attacking a high percentage of fruit if no control measures are applied.

(2) The pest attacks the crop shortly before harvest time, rendering chemical control very difficult.

(3) Although chemical control of the cherry fruit fly is effective there is the danger of undesirable pesticide residues due to the need of spraying close to harvest time and the danger of developing pesticide resistance.

Feasibility of the sterile-insect release method for R. cerasi

(1) The topographical situation in Central Europe is most favourable for this type of control (natural island situations due to mountains and forests). Experimental areas of all sizes are available.

(2) The population fluctuations of R. cerasi show generally 4-5 year periods of high population density, followed by about equal periods of extremely low population densities. This phenomenon apparently can be observed in the whole Central European area at about the same time.

(3) The pupal diapause could be used for rearing the fly on a 52-week basis and the pupal material could be accumulated until used during 2-4 weeks each year. Thus relatively small rearing facilities would be required.

(4) The short flight range of R. cerasi (average 100-200 m) is another advantage in regard to reinfestation.

(5) All of these factors indicate that this species has unique advantages for the development and application of the sterile-insect release method.

Recommendations

It is recommended that:

(a) That Joint FAO/IAEA Division should stimulate research in the sterile-insect release method for this species and attempt a co-ordination of research between interested laboratories, establishing research contracts and agreements. Provisions should be made for a continuous flow of information between workers on this species and specialists working on other fruit fly species.

(b) More ecological information is required. More demographic field studies should be undertaken in order to provide the necessary data for a thorough evaluation of this method of insect control and its eventual integration with other control methods. Data listed in Annex B should be derived as the sterile-insect release method for control or eradication of R. cerasi progresses.

ANNEX A

PROPOSED EXPERIMENT TO ERADICATE THE MEDITERRANEAN FRUIT FLY BY THE STERILE-MALE TECHNIQUE FROM NICARAGUA

I. INTRODUCTION

1. During the past few years it has been shown that the sterile-male technique, either alone or as part of an integrated program with insecticides, can be utilized to eradicate the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). However, no large-scale eradication experiment has been carried out, primarily because of the high cost.

2. The medfly was introduced into Central America before 1955. Since its discovery in Costa Rica in 1955 it has spread throughout Costa Rica, southern Nicaragua, and western Panama. Unless it is eradicated,

there is no doubt that it will eventually spread throughout Panama, as well as northward into other Central American countries, Mexico, and the USA, resulting in tremendous economic losses.

II. RECOMMENDATIONS

3. It is recommended that a large-scale medfly eradication experiment be conducted, in Nicaragua, for the following reasons:
   (a) The medfly-infested area of Nicaragua is well isolated geographically (a minimum of quarantine procedures would be necessary to prevent reinfestation from the south (Costa Rica)), and no known infestation exists north of Nicaragua;
   (b) If successful, the northward spread of the medfly would be stopped;
   (c) Data from the active program (funded under UNDP(SF) and executed by the IAEA) carried out in Central America to develop the sterile-male technique for medfly eradication support the conclusion that the medfly can be eradicated in Central America, using this technique.

It is further recommended that the medflies required for this experiment be reared and sterilized in Costa Rica and subsequently air-shipped to Nicaragua, thus avoiding the expense of constructing a medfly escape-proof rearing plant in Nicaragua.

III. OBJECTIVES

4. The objectives of the proposed large-scale experiment would be to:
   (a) Develop and demonstrate procedures for medfly eradication by a sterile-insect-release/insecticide-application integrated program for use in developing countries;
   (b) Establish a realistic cost basis for medfly eradication using the sterile-male technique plus insecticides;
   (c) Stop the northward spread of the medfly;
   (d) Conduct research to further improve the effectiveness and economy of the sterile-male technique for medfly eradication.

IV. GENERAL OUTLINE OF THE PROPOSED EXPERIMENT

5. Although the actual size of the medfly infested area in Nicaragua is not known, estimates range from about 1300 to 4000 km^2 (the latter figure should be used until it is proven incorrect). Nearly all the infested terrain is mountainous; thus the actual area, based on row feet of crops, may be considerably larger than estimated. Coffee is by far the predominant host plant; little citrus of commercial value is grown. The primary medfly hosts, other than coffee, include sweet oranges, mandarines, grapefruit, and several wild hosts.

6. Before the project operations are actually initiated the following should be completed:
   (a) Plan of all details, objectives, and target dates, using the PERT system;

(b) Construction plans for a completely new medfly rearing plant with a capacity of 500 million flies/week (a consultant with experience in designing similar facilities should be employed for this);

(c) Purchase or lease of land for the location of the plant to be constructed not less than 11 km from the San José, Costa Rica airport;

(d) Hire of the following professional staff:

    (i) The project manager;

    (ii) A rearing supervisor;

    (iii) An ecologist/biometrician;

    (iv) An aerial release supervisor;

    (v) A survey/assessment/quarantine supervisor;

and the following non-professional staff:

    (vi) An administrative aid;

    (vii) A secretary;

    (viii) Several laboratory technicians, particularly for the rearing plant;

(e) Preparation of the list of equipment and supplies required for the first year of operation (PERT input data);

(f) Obtaining of suitable office and laboratory facilities in or near Managua, Nicaragua, for project headquarters.

7. First year:

(a) Construct and equip a new, 500 million/week-capacity medfly rearing plant in San José, Costa Rica; plant to be partially fly escape-proof;

(b) Conduct an intensive medfly population survey in Nicaragua to delineate the actual infested area (trapping operation);

(c) Conduct field cage tests to evaluate the optimum radiation sterilization dose;

(d) Conduct research to develop a better and cheaper method for the aerial release of sterile flies, an adequate method of insecticide application for medfly population suppression and a satisfactory guidance system for release and insecticide application aircraft;

(e) Develop trapping and assessment techniques for relatively inaccessible areas (investigate the use of helicopters);

(f) Develop an absolute method of marking sterile flies;

(g) Eliminate medfly infestations in Jinotega, Corinto, and in other isolated locations (if any) north of Managua using insecticides and sterile flies.

8. Second year:

(a) Increase the production of medflies to the maximum rearing plant capacity;

(b) Apply insecticide to reduce medfly populations in the northern part of the infested area, that is, in areas of high infestation;

(c) Initiate releases of sterile medflies in the northern part of the infested area, at appropriate time intervals following insecticide applications;

(d) Establish quarantine procedures at the Nicaragua-Costa Rica border;

(e) Continue the survey (see para. 7b above);

(f) Finalize the guidance system for the aerial release of sterile insects and the application of insecticide sprays.

9. Third year:
(a) Release sterile flies over the entire infested area following insecticide applications, if needed;
(b) Regularly assess the effectiveness of procedures, develop necessary refinements and determine project achievements;
(c) Continue survey and quarantine operations.
10. Fourth year:
(a) Continue survey and quarantine operations;
(b) Continue assessment procedures;
(c) Carry out clean-up operations.

V. DESCRIPTION OF SPECIFIC ACTIVITIES

A. Survey

11. The medfly population survey to be conducted during the first year of the project should be made utilizing disposable sticky traps and not the more expensive plastic traps. The traps, baited with trimedlure, would be serviced about every 2 weeks. The traps must be monitored continuously over at least a six-month period (December through May), but preferably over a ten-month period (October through July). Owing to the inaccessibility of many areas (which may be infested), it is important that traps be carefully located in known host areas. Furthermore, traps must be placed in all communities accessible by jeep. Other high priority areas for trapping include (i) along the Rio San Juan, from Lake Nicaragua to the Caribbean Sea, (ii) communities along the Caribbean Sea, (iii) communities not accessible by jeep but accessible by horse, (iv) areas known previously to have been infested, e. g. Corinto and Jinotega (these areas must be intensively trapped), (v) islands in Lake Nicaragua and Lake Managua, and (vi) along all railroad tracks. Traps can be located in areas accessible only by horse either by using a small helicopter to drop large, well-marked sticky traps or by employing local residents (i.e. trappers on horseback) to operate these traps.
12. Trap density is directly related, within limits, to the probability of detecting a medfly infestation. It is suggested that about one trap per km^2 be used in the 4000 km^2 area which could be infested. In other areas, traps should be located along roads, in communities, etc., as described above.

B. Rearing plant

13. The rearing plant should be designed with the maximum amount of automated equipment that can be adequately serviced. The provision of an auxiliary power supply is mandatory; the installation of exhaust fans for cooling and electric heaters from heating must be adequate to ensure the maintenance of the correct ambient temperature. The equipment should include automatic devices for egg collection and the separation of pupae from the preparation media, and semi-automatic devices for media separation, the packaging of pupae and irradiation.
14. The partial fly escape-proofing of the plant should include: (i) building construction foreseeing at least double- and preferably

triple-stage entrance and exit doors, (ii) sealed windows, (iii) a sewage system capable of handling huge amounts of spent media or a high temperature room for killing all larvae and pupae left in the media, (iv) one-way traffic flow for all supplies — all expendable materials leaving the plant, except for sterile flies, are to be heated to a temperature high enough to kill all medflies, irrespective of stage of development, should they be on or in these materials, and (v) plant construction design permitting absolute fly escape-proofing with minimum renovation.

15. The location of the rearing plant in San José, Costa Rica, must be fairly close to the airport but not so close that escaped flies can 'hitchhike' on aircraft. Therefore, the plant should be no closer than 11 km from the airport. Furthermore, several million sterile flies should be released weekly within a 3 km radius of the rearing plant.

16. A consultant experienced in the design of similar facilities should be employed to design and oversee construction of this plant. However, at least one entomologist experienced in medfly mass rearing should work closely with the consultant.

C. Release system for sterile flies[1]

17. The cost ($0.054 each) of the present release container, which consists of a paper sack containing excelsior and a food wick, must be reduced. More than 50% of this cost is for the excelsior. If the excelsior cost can be reduced one-half, perhaps through the use of a cheaper but adequate substitute, then the total cost of each release container could be reduced by about 25%.

18. In the proposed experiment the release rate of sterile flies can be lower than during the previous project: by using insecticides and sterile flies it is anticipated that the release rate need only be 1000 sterile flies/100 m, as compared to 5000/100 m of flight line, in the previous project. Accordingly, it is expected that the release container can be reduced to one-third of its present size; thus the C-47 payload could be tripled, that is, from 2500 to 7500 containers per flight. Further, the cost per container undoubtedly could be reduced to about $0.02. Thus, to release 300 million sterile flies per week would require 300 000 containers costing an estimated $6000.

19. The release of free pupae (without any container) has shown promise in preliminary experiments conducted in Hawaii and Costa Rica. These studies indicate that the release of free pupae is about 25% to 40% as efficient as the release of adults in containers. The release of free pupae rather than adult flies offers several advantages, including: much lower container costs; the fact that far fewer personnel and less equipment are required to package pupae; a much higher payload for the release aircraft; the possibility of using single-engine airplanes which cost much less to operate than C-47 aircraft ($25/h as compared to $100/h); and a more uniform distribution of the sterile insects. Disadvantages of the free pupae release method include the following points: it is difficult to time the time interval between irradiation and release (i.e. to ensure that flies do not emerge before or during release operations);

---

[1] Additional data are given in the Appendix to this Annex.

pupae must be irradiated, whereas with the container release system
either pupae or adults can be irradiated if a "food" type irradiation source
is available; the number of effective sterile flies/unit area may be some-
what more variable because free pupae falling in non-shaded areas would
probably perish; and the number of flies to be reared would have to be
increased 2 or 3 times, thereby increasing the cost of plant operation.

D. Insecticide applications

20. Although considerable data are available on insecticide-bait
treatments for medfly control, additional information would be required
for the proposed experiment in Nicaragua, particularly in using the
ultra low volume method (toxicant plus bait without any carrier). Multi-
engine aircraft with auxiliary power for jet-assisted take-off probably
would be necessary because of the rough terrain and turbulent weather
conditions. The assistance of the USAF should be requested in this
regard.

21. Other factors which should be evaluated include: (i) a comparison
between malathion plus bait and Lebaycid plus bait; (ii) the dosage of
toxicant required to realize 95-98% killing of adult medflies; (iii) the
optimum ratio of toxicant to bait; (iv) whether it is necessary to spray
the entire area or only every second or third swath; (v) the duration of
residual effectiveness of spray applications to determine maximum
allowable interval between applications; and (vi) guidance system develop-
ment, as discussed in section E below.

22. The overall objectives of the insecticide-bait applications would
be threefold: (i) to reduce the wild-fly population to a low level (to about
1000 to 1500 wild flies/km^2), where necessary, before or at the beginning
of the sterile-fly release operations in a given area; (ii) to knock out 'hot
spot' infestations once release of sterile flies has begun; and (iii) to
create a barrier between the present infested area and sections of the
country further north.

23. It is estimated that an area of approximately 3500 km^2 will
require insecticide application.

E. Aerial guidance system

24. In an experiment such as this where precise dispersal of the
released insects is of prime importance, a reliable system of guiding
the release aircraft is essential; furthermore, aircraft dispersing insecti-
cides must be accurately guided to ensure adequate spray patterns. Sterile
flies should be released 75 m to 100 m above ground level (unless a better,
high-altitude release method is developed) and spray aircraft should fly
about 30 m above ground level. The use of helium-filled balloons as a
guidance system may be feasible in certain areas; however, the strong
winds, rough terrain and extensive shade tree cover grown over coffee
will dictate another method in most cases. Two alternatives appear
possible: (i) use of the 'Delta' system of direct radio-guidance, or
(ii) use of spotter aircraft to guide the release and spray plants. Since
the 'Delta' system (which has been extensively used in the USA for large-
scale insecticide spraying on level terrain) depends on 'line-of-sight'
radio contact, the rough mountainous terrain in Nicaragua may make

this system non-workable. 'Delta' representatives, however, should
be contacted before rejecting the possible use of this system. The
use of spotter aircraft in conjunction with a ground marking system, on
the other hand, may prove the more effective guidance system.

F. Marking of sterile flies

25. To evaluate accurately certain aspects of a sterile-insect
release program, a reliable method of marking the released insects is
necessary. The present method of marking sterile medflies is based
on mixing the pupae with a fluorescent dye powder; when the adults
emerge they pick up sufficient dye, particularly in their ptilinum, to
distinguish them from unmarked flies. Unfortunately, only 95% to 99%
of the adults become marked by this method, whereas 100% marking
efficiency is desirable. Trapped marked flies are used to estimate the
ratio of sterile to wild flies and thus constitute the source of data for
special studies on longevity, flight distance, distribution patterns, etc.
During the initial phases of this experiment a concerted effort should
be made to develop a method of marking which is simple and 100%
effective; this should include attempts to improve on the present methods:
use of dyes in solution, incorporating dyes in the larval diet, and develop-
ing genetic markers.

VI. PROJECT STAFF

26. The staff employed for this experiment must be of high calibre,
well qualified, have a good understanding of the principles and problems
involved in the sterile-male technique, should have a good command of
or be willing to learn Spanish, and be willing to follow the direction of
the project manager. Also, it is strongly recommended that a five-man
steering committee be appointed to oversee the entire operation; the
project manager would report directly to this committee on technical
matters. At least three members of this committee should be nationals
of countries from outside of Latin America. All of the committee mem-
bers must have a good working knowledge of the sterile-male technique
and have had experience in large field programs to eradicate insects
(preferably using sterile-male technique). The committee should visit
the project site and hold program review meetings at least every 3-4
months.

A. Staff required in Nicaragua (80):

27. Professionals (5):
(a) The project manager;
(b) An assistant manager, responsible for insecticide applications;
(c) A survey, assessment, and quarantine supervisor;
(d) An aerial release supervisor;
(e) A research ecologist/biometrician;
28. Non-professionals (75):
(a) Survey, assessment and quarantine staff (45):
   (i) Technicians (6);
   (ii) Trappers (24);

(iii) Quarantine helpers (9);
(iv) Speciality unit helpers (4);
(v) An auto mechanic;
(vi) A mechanic helper;
(b) Aerial release staff (14):
(i) Technicians (6);
(ii) Helpers (8);
(c) Ecology/biometrics staff (9):
(i) Technicians (7);
(ii) Helpers (2);
(d) Office staff (7):
(i) Secretaries (2);
(ii) Administrative assistants (2);
(iii) Typists (2);
(iv) A janitor.

B. Staff required in Costa Rica (42):

29. Professionals (3):
(a) A rearing supervisor;
(b) A quality control supervisor;
(c) A rearing research specialist;
30. Non-professionals (39):
(a) Technicians (14):
(i) Rearing and handling (10);
(ii) Rearing research (2);
(iii) Quality control (2);
(b) Laboratory helpers (20);
(c) Janitors (2);
(d) A maintenance supervisor;
(e) A maintenance helper;
(f) A secretary.
31. The total thus required, from sections A and B above, is 122,
made up as follows:
(a) Professionals (8);
(b) Non-professionals (114).

VII. ESTIMATED COSTS

32. Staff: $ 1 535 600

(a) Professionals (8):
    five, $ 27 100 each/yr, 4 years; $ 542 000
    three, $ 25 800 each/yr, 4 years; $ 309 600
(b) Non-professionals (114):
    $ 1 500 each/yr, 4 years $ 684 000

33. <u>Facilities:</u> $ 650 000

(a) Costa Rica:
    (i) Rearing plant[2] and office $ 250 000
    (ii) Rearing plant equipment $ 250 000
    (iii) Storage building $ 20 000
    (iv) Land (cost of leasing
        5 acres) $ 20 000
                             $ 540 000

(b) Nicaragua:
    (i) Office and laboratory space $ 100 000
    (ii) Mechanics shop $ 10 000
                             $ 110 000

34. <u>Medfly production:</u> $ 450 000[3]

(a) 350 000 000 flies/week, $ 400 000
    $ 5 250/week, 75 weeks
    (rearing diet costs $ 15/million)
(b) Contingency fund $ 50 000

35. <u>Insecticide applications (including guidance costs):</u> $ 525 000

Cost = $ 150/km^2; 3 500 km^2 to be
treated (see feasibility report
for details)

36. <u>Equipment and supplies (other than for rearing):</u> $ 591 000

(a) Vehicles (30) $ 150/month, 48 months $ 216 000
(b) Miscellaneous laboratory equipment $ 50 000
(c) Field equipment (traps, trimedlure,
    weather stations, radios for
    vehicles, etc.) $ 200 000
(d) Miscellaneous supplies $ 50 000
(e) Quarantine equipment and supplies $ 50 000
(f) Office supplies and equipment
    (including communications) $ 25 000

---

[2] A fly 'escape-proof' rearing plant is estimated to cost $500 000 to $750 000.

[3] This amount would increase at least twofold if the free pupal release method were used; see footnotes 4 and 5.

37. <u>Aircraft costs</u>: $ 1 550 000[4]

(a) Helicopter for survey and assessment
    flights: $ 200/h; 5 h/day, 200 days $ 200 000
(b) Release aircraft: $ 1 350 000
    Assuming the release area is 4 000
    km^2 and flight lines are 400 m apart,
    the total length of flight lines for the
    area would be 10 000 km. A minimum
    of three releases/week would be
    required, making a total of 30 000 km
    over the release area. As the air-
    craft flies 200 km/h (C-47), the
    release time would be 150 h/week,
    plus time to turn and time to fly to
    and from the release area. Total
    flight time/week is estimated at
    325 h; cost is $ 100/h, thus
    $ 22 500/week, 60 weeks.

38. <u>Cost of release containers</u>:[5] $ 360 000

    The cost of the smaller release container
    being proposed is estimated at $ 0.02 each.
    Releases would be made at the rate of
    one container/100 m along the flight
    lines. Each flight over the entire
    area consists of 10 000 km (10 000 000 m),
    thus would require 100 000 release units
    costing $ 2 000; three releases/week thus
    would cost $ 6 000; the containers for
    a 60-week program would thus cost
    an estimated $ 360 000.

39. <u>Sub-contracted research</u>: $ 200 000

(a) IAEA Seibersdorf Laboratory: supplies,
    equipment and personnel $ 100 000
(b) USDA Fruit Fly Laboratory Hawaii:
    supplies, equipment, and personnel $ 100 000

---

[4] Using the free pupal release method, the release aircraft cost per hour could be reduced to $25.
If three times the usual number of insects were released (thus, 900 million pupae/week), the aircraft cost
would be $15 000/week for the releases plus the cost of ferrying pupae from San José to Managua. This
latter cost is estimated at 36 hours/week at $100/h or a total of $216 000 for 60 weeks. Therefore, the
free pupal release method would cost a total of about $1 116 000. In addition, there would be higher
rearing costs to be met (see para. 34).

[5] The use of the free pupal release method would essentially eliminate this cost (see para. 34).

40. Summary of costs

(a)	Staff	$ 1 535 600
(b)	Facilities	650 000
(c)	Medfly production	450 000
(d)	Insecticides	525 000
(e)	Equipment and supplies	591 000
(f)	Aircraft	1 550 000
(g)	Release containers	360 000
(h)	Research	200 000
	Sub-total	$ 5 861 600
(i)	Contingency fund	100 000
	Total	$ 5 961 600

APPENDIX TO ANNEX A

Required release rate (flies per week) to obtain various ratios of sterile to wild flies, based on infested areas of three different sizes and varying wild fly populations

Infested area in km²	Population of wild flies per km²	Total population in the infested area (× 10⁶)	Required release rate per week (flies/week × 10⁶) to obtain the indicated ratio of sterile to wild flies, assuming that 50% of the released flies are lost[a]			
			25:1	50:1	100:1	200:1
4000	1000	4	200	400	800	1600
4000	2000	8	400	800	1600	3200
4000	4000	16	800	1600	3200	6400
3000	1000	3	150	300	600	1200
3000	2000	6	300	600	1200	2400
3000	4000	12	600	1200	2400	4800
2000	1000	2	100	200	400	800
2000	2000	4	200	400	800	1600
2000	4000	8	400	800	1600	3200

[a] If the free pupal release method is used the release rate probably must be doubled or tripled to compensate for the additional loss of flies.

ANNEX B

ADDITIONAL INFORMATION REQUIRED ON FRUIT FLIES

The following are some of the subjects on which additional information should be obtained to increase the efficiency and lower the cost of the sterile-insect release method for species for which this technique is applicable.

1. Mass rearing (1 000 000 per week or more)

    (a) Standardization of diets and nutritional studies resulting in a
        more efficient production and improvement in quality of the
        insect with respect to the following characteristics: vitality,
        sexual aggressiveness, longevity, etc.
    (b) Introduction of new strains of wild populations into existing
        mass-reared strains.
    (c) Study of symbionts and their importance.
    (d) Improvement and possible mechanization of rearing devices to
        reduce costs.

2. Irradiation.   Improvement of irradiation technology including both
physical and biological characteristics.   Sterilization studies including
determination of optimum radiation dosage in relation to maximum
competitiveness in laboratory and field experiments, should be performed.

3. Marking.   At present, irradiated flies are usually marked with
fluorescent dyes before release.   The use of alternative methods of
marking (including isotopes and genetic markers) may prove useful.

4. Quality control.   Improvement of quality control measures and
development of fail-safe methods to guarantee effective irradiation
concurrent with a continuing supply of high-quality insects.

5. Release methods

    (a) Improved methods of feeding of adults in release containers;
        determination of need for this.
    (b) Evaluation of pupal versus adult serial releases.
    (c) Improvement of release containers and reduction of cost.

6. Survey, sampling and evaluation techniques

    (a) Improvement of trapping methods and correlation of trap catches
        to population density and infestation.
    (b) Improvement and standardization of assessment techniques
        including fruit sampling and hatchability of field collected eggs.
    (c) Chemical and physical attractants.

7. Biology and ecology

    (a) The limits of the distribution of any species should be accurately
        determined before eradication is attempted.
    (b) Population dynamics including density, fluctuations, dispersal,
        and seasonal and climatic effects must be studied.
    (c) Behaviour of wild and released flies including oviposition,
        feeding, larval and pupal reactions, and expecially sexual
        behaviour in the laboratory and field throughout the adult life
        should be studied.
    (d) Quantitative life cycle tables should be calculated.

8.   Adverse effects. Investigation of punctures on fruit caused by irradiated females that may adversely affect their commercial value should be determined.

9.   Economic aspects

(a) Economic damage caused in the countries or areas where the sterile-insect release method is considered should be determined as well as the status and cost of current control practices.

(b) Data on the actual cost of the application of the method on a large-scale basis, either alone or as a part of an integrated approach, are evidently lacking. Present research and experimentation has been directed mainly towards proving the feasibility of the method from a purely technical point of view and restricted to small areas. No realistic cost estimates can be available until larger-scale experiments are carried out and properly evaluated.

(c) Correlation and development of an integrated control program including the sterile-insect release method is required.

10. Genetic studies. Basic research on fruit-fly genetics, especially in the area of altering the sex ratio of colony strains and also developing other methods of inducing sterility should be investigated.

LIST OF PARTICIPANTS

CHAMBERS, D.L.
Fruit Fly Laboratory,
P.O. Box 2280,
Honolulu, Hawaii 96804,
United States of America

CHEIKH, M.
Entomology Department,
National Institute of Agricultural
Research,
Ariana, Tunisia

CIRIO, U.
Comitato Nazionale per l' Energia
Nucleare,
Centro di Studi Nucleari della Casaccia,
S.F. Anguillarese km 1+300,
Rome, Italy

HAISCH, A.
Bayerische Landesanstalt für
Bodenkultur, Pflanzenbau und ·
Pflanzenschutz,
Menzingerstrasse 54,
8 Munich 19,
Federal Republic of Germany

KATIYAR, K.P.
Interamerican Institute of Agricultural
Sciences,
Turrialba, Costa Rica

LOPEZ D., Fernando
Caribbean Fruit Fly Investigations,
United States Plant Protection Station,
13601 Cutler Road,
Miami, Florida 33158,
United States of America

MELLADO, L.
National Institute of Agronomical
Research,
Agricultural Phytopathology Station,
Madrid, Spain

MEWS, A.
Department of Zoology,
University of Edinburgh,
West Mains Road,
Edinburgh 9,
United Kingdom
(Present address: International
Atomic Energy Agency, Vienna)

MOURIKIS, P.

Benaki Phytopathological Institute,
Kifissia,
Athens, Greece

de MURTAS, I.

Comitato Nazionale per l'Energia
Nucleare,
Centro di Studi Nucleari della Casaccia,
S.F. Anguillarese km 1+300,
Rome, Italy

REY, J.

Instituto Español de Entomología,
J. Gutiérrez Abascal 2,
Madrid 6, Spain

RUSS, K.

Federal Institute for Plant Protection,
Trunnerstrasse 5,
1020 Vienna, Austria

SILVA, G. de Magalhães

Estação Agronómica Nacional,
Depto. de Entomología,
Oeiras, Portugal

YEN, D.F.

Division of Plant Industry,
Joint Commission of Rural
Reconstruction,
37 Nan Hai Road,
Taipei, Taiwan,
Republic of China

Representatives of International Organizations

BERG, G.H.

Plant Production and Protection
Division,
Food and Agriculture Organization of
the United Nations,
Via delle Terme di Caracalla,
Rome, Italy

BOLLER, E.F.

Swiss Federal Research Station for
Agriculture, Viticulture and
Horticulture,
CH 8820 Wädenswil,
Switzerland
(member of International Organization
for Biological Control of Noxious
Animals and Plants, Zürich,
Switzerland)

CAVALLORO, R.   Comunitá Europea dell' Energia
                                    Atomica,
                                 Ispra, Italy

PAL, R.   World Health Organization,
                                 1211 Geneva 27,
                                 Switzerland

International Atomic Energy Agency

HOOPER, G.H.S.   Division of Research and
                                    Laboratories
LaCHANCE, L.E.   Joint FAO/IAEA Division of Atomic
                                    Energy in Food and Agriculture
LANGLEY, P.A.   Division of Research and
                                    Laboratories
LINDQUIST, D.A.   Joint FAO/IAEA Division of Atomic
                                    Energy in Food and Agriculture
NADEL, D.J.   Division of Research and
                                    Laboratories
OUYE, M.T.   Joint FAO/IAEA Division of Atomic
                                    Energy in Food and Agriculture

RHODE, R.H.   IAEA Expert, Apartado Postal 3628,
                                 San José, Costa Rica

Scientific Secretary

ENKERLIN S., D.   Joint FAO/IAEA Division of Atomic
                                    Energy in Food and Agriculture

IAEA SALES AGENTS AND BOOKSELLERS

Orders for Agency publications can be placed with your bookseller or any of the addresses listed below:

ARGENTINA
Comisión Nacional de
Energía Atómica
Avenida del Libertador 8250
Buenos Aires

AUSTRALIA
Hunter Publications
23 McKillop Street
Melbourne, C.1

AUSTRIA
Publishing Section
International Atomic Energy Agency
Kärntner Ring 11
P.O. Box 590
A-1011 Vienna

BELGIUM
Office International
de Librairie
30, Avenue Marnix
Brussels 5

CANADA
Information Canada
Ottawa, Canada

C.S.S.R.
S.N.T.L.
Spolena 51
Nové Mesto
Prague 1

DENMARK
Ejnar Munksgaard Ltd.
6 Norregade
DK-1165 Copenhagen K

FRANCE
Office International de
Documentation et Librairie
48, Rue Gay-Lussac
F-75 Paris 5e

HUNGARY
Kultura
Hungarian Trading Company
for Books and Newspapers
P.O. Box 149
Budapest 62

INDIA
Oxford Book & Stationery Comp.
17, Park Street
Calcutta 16

ISRAEL
Heiliger & Co.
3, Nathan Strauss Str.
Jerusalem

ITALY
Agenzia Editoriale Commissionaria
A.E.I.O.U.
Via Meravigli 16
I-20123 Milan

JAPAN
Maruzen Company, Ltd.
P.O. Box 5050,
100-31 Tokyo International

MEXICO
Librería Internacional, S.A.
Av. Sonora 206
México 11, D.F.

NETHERLANDS
Martinus Nijhoff N.V.
Lange Voorhout 9
P.O. Box 269
The Hague

PAKISTAN
Mirza Book Agency
65, Shahrah Quaid-E-Azam
P.O. Box 729
Lahore - 3