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

EFFECTS OF IONIZING RADIATION ON INSECTS AND OTHER ARTHROPODS

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Abstract — Résumé — Аннотация — Resumen

EFFECTS OF IONIZING RADIATION ON INSECTS AND OTHER ARTHROPODS. Research into the possible application of the radiation sterilization method of population suppression is now under way on a number of insects that attack man, animals and a variety of crops. These exploratory investigations have shown that ionizing irradiation will induce sterility but there is considerable variation in the amounts needed. The research also suggests that radiation damage may in some cases prevent application of the method to some insects. A frequent obstacle that must be overcome is lack of practical mass-rearing methods. Some insects also appear to be so abundant that the use of the technique may not be feasible without first processing the infested area with other control measures to bring wild populations within reach. Despite these difficulties, when conditions are favourable, few other approaches to the control of pests are so potentially rewarding. The radiation sterilization method may also be thought of as a possible means of delaying development of infestation until crops are harvested.

The present paper reports on the influence of gamma radiation on the reproductive potential, sexual aggressiveness, vigour and longevity of the oriental fruit fly, Dacus dorsalis Hendel, the melon fly, Dacus cucurbitae Coq., the Mediterranean fruit fly, Ceratitis capitata Wied., the Mexican fruit fly, Anastrepha ludens Loew, and Anopheles quadrimaculatus Say, and results of practical field trials of the sterile-male release method of population suppression. Progress in the campaign to eradicate the screw-worm, Cochliomyia hominivora Coql., in the United States and in studies to develop vigorous genetically marked strains that will permit ready identifications of released sterile flies is reviewed. Results of irradiation research on six additional species that infest fruit, vegetable, field and forest crops, three that attack livestock, three that largely affect man, the effects of irradiation on the scorpion, Centruroides limpidus Karsch, and the Lone-Star tick, Amblyomma americanum L., and ionizing radiation as a possible quarantine treatment for fruits and vegetables infested with fruit flies and mangoes infested with the mango weevil, Sternochetus mangiferae Fabricius, are also discussed.

EFFET DES RAYONNEMENTS IONISANTS SUR LES INSECTES ET AUTRES ARTHROPODES. Des recherches sur la possibilité d'appliquer la méthode de stérilization par les rayonnements pour l'éradication de populations d'insectes sont actuellement en cours pour un certain nombre d'insectes nuisibles à l'homme, aux animaux et à diverses cultures. Il ressort de ces recherches préliminaires que les rayonnements ionisants provoquent la stérilité, mais que les doses nécessaires varient considérablement. Ces recherches donnent aussi à penser que les dommages radioinduits sont tels qu'il ne serait pas opportun d'appliquer cette méthode à toutes les espèces d'insectes. Une difficulté à laquelle on se heurte fréquemment est l'absence de méthodes pratiques d'élevage en masse. D'autre part, certaines populations d'insectes sont tellement nombreuses qu'il peut être impossible de recourir à cette méthode sans avoir au préalable appliqué dans la région infestée d'autres méthodes de lutte de manière à pouvoir atteindre les populations naturelles. Malgré ces difficultés, si les conditions sont favorables, il existe peu d'autres méthodes qui puissent donner d'aussi bons résultats. On peut aussi envisager de recourir à la méthode de stérilisation par les rayonnements pour retarder la pullulation des insectes jusqu'à la moisson des récoltes.

Le mémoire rend compte de l'influence des rayons gamma sur la capacité de reproduction, l'agressivité sexuelle, la vigueur et la longévité de divers insectes: mouche orientale des fruits Dacus dorsalis Hendel, mouche du melon Dacus cucurbitae Coq., mouche méditerranéenne des fruits Ceratitis capitata Wied, mouche mexicaine des fruits Anastrepha ludens Loew et Anopheles quadrimaculatus Say; il donne aussi les résultats d'expériences réelles de lâcher de mâles stériles pour l'éradication des insectes. L'auteur analyse les résultats obtenus dans la campagne d'éradication de la lucilie bouchère Cochliomyia hominivorax Coql. aux Etats-Unis, ainsi que dans des études visant à produire des souches vigoureuses, marquées génétiquement en vue d'identifier

facilement les mouches stériles après leur lâcher. Il étudie aussi les résultats de recherches sur différents sujets: effets des rayonnements sur six autres espèces nuisibles aux fruits, aux légumes, aux cultures et aux forêts, sur trois espèces qui s'attaquent au cheptel et sur trois espèces qui sont particulièrement nuisibles à l'homme; effets des rayonnements sur le scorpion Centruroides limpidus Karsch et sur la tique Amblyomma americanium L.; possibilité d'utiliser les rayonnements ionisants pour traiter - comme mesure de quarantaine - les fruits et légumes attaqués par les mouches des fruits et les mangues attaquées par le charançon de la mangue Sternochetus mangiferae Fabricius.

ВОЗДЕЙСТВИЕ ИОНИЗИРУЮЩЕЙ РАДИАЦИИ НА НАСЕКОМЫХ И ДРУГИХ ЧЛЕНИСТОНОГИХ. В настоящее время проводятся исследования возможности применения метода стерилизации посредством облучения для уничтожения популяций целого ряда насекомых, поражающих человека, животных и различные культуры. Эти предварительные исследования показали, что ионизирующее облучение приводит к стерилизации, но что для этой цели требуются чрезвычайно разнообразные дозы. Оказалось, что в некоторых случаях радиационные повреждения могут исключить возможность применения этого метода у некоторых насекомых. Препятствие, которое зачастую приходится преодолевать, заключается в отсутствии практических методов разведения насекомых в массовых количествах. С другой стороны, некоторые виды насекомых являются настолько многочисленными, что применение этого метода может оказаться неосуществимым без предварительной обработки зараженного района другими средствами истребления для привлечения на доступное расстояние популяций насекомых. Несмотря на эти затруднения, при благоприятных обстоятельствах вряд ли какие-либо другие методы истребления вредных насекомых представляются столь же перспективными. Метод стерилизации при помощи радиации может служить способом отдаления инвазии до окончания сбора урожая.

В докладе сообщается о воздействии гамма-излучения на потенциал размножения, половую агрессивность, силу и живучесть восточной плодовой мухи Dacus dorsalis (Hendel), дынной мухи Dacus cucurbitae (Coq.), средиземноморской плодовой мухи Ceratitis capitata (Wied.), мексиканской плодовой мухи Anastrepha ludens (Loew) и малярийного комара Anopheles quadrimaculatus (Say); в докладе приводятся также результаты практических испытаний выпуска стерилизованных самцов на опытное поле для истребления популяций. Дается описание дальнейшего хода кампании по истреблению бычьего овода Cochliomyia hominivorax (Coqrl.) в Соединенных Штатах Америки и изучения разведения сильных, генетически маркированных особей, которые позволят легко выявлять выпущенных на волю стерилизованных оводов. Обсуждаются также результаты исследований по облучению 6 дополнительных видов вредителей, поражающих фрукты, овощи, полевые и лесные культуры, 3 видов, поражающих скот, и 3 видов, нападающих главным образом на человека, а также воздействие облучения на скорпионов Centruroides limpidus (Karsch), одновездчатого клеща Amblyomma americanum (L.); далее обсуждается возможность использования ионизирующего излучения в качестве возможного гарантийного средства для дезинфестации овощей и фруктов, пораженных плодовой мухой, а также манго, пораженного мангровым долгоносиком Sternochetus mangiferae (Fabricius).

EFECTOS DE LAS RADIACIONES IONIZANTES SOBRE LOS INSECTOS Y OTROS ARTRÓPODOS. Actualmente se estudia la posibilidad de recurrir a la radioesterilización para exterminar a diversos insectos que atacan al hombre, a los animales y a las plantas. Las primeras investigaciones han demostrado que las radiaciones ionizantes producen esterilidad, y que las dosis necesarias varían considerablemente. También hacen pensar que los daños producidos por las radiaciones pudieran en algunos casos impedir que se aplique el método a ciertos insectos. Con frecuencia, el obstáculo principal es la falta de métodos de cría de los insectos en gran escala. En otros casos, el insecto se da con tal abundancia que no es posible aplicar esta técnica sin antes tratar por otros medios la zona infestada a fin de reducir el número de insectos. A pesar de todas las dificultades, el método de la radioesterilización es uno de los mejores, cuando las condiciones son favorables. También se puede utilizar para retrasar el desarrollo de la plaga hasta después de la cosecha.

La memoria informa sobre la influencia de las radiaciones gamma en la capacidad de reproducción, los instintos sexuales, el vigor y la longevidad de la mosca oriental de la fruta, Dacus dorsalis Hendel, la mosca del melón, Dacus cucurbitae Coq., la mosca mediterránea, Ceratitis capitata Wied., la mosca de la fruta mejicana, Anastrepha ludens Loew, y el Anopheles quadrimaculatus Say, e indica los resultados de la campaña de exterminio obtenidos en la práctica liberando machos estériles. Revisa también los progresos realizados en los Estados Unidos en la campaña para exterminar la Cochliomya hominivorax Cqrl., y en los estudios para desarrollar cepas vigorosas, marcadas genéticamente, que permitan identificar con facilidad las moscas estériles liberadas. Se discuten también los resultados de las investigaciones sobre la irradiación de

otras seis especies que atacan a frutas, verduras y otros cultivos agrícolas y forestales, otras tres que atacan al ganado, y tres más que atacan principalmente al hombre. Se trata asimismo de la irradiación del escorpión, Centruroides limpidus Karsch y del arácnido Amblyomma americanum L., y de la posibilidad de emplear radiaciones ionizantes como tratamiento de cuarentena para las frutas y verduras infestadas con la mosca de la fruta y para los mangos infestados con el gorgojo Sternochetus mangiferae Fabricius.

1. INTRODUCTION

The eradication of the screw-worm (Cochliomyia hominivorax Coquerel) from the island of Curaçao by the release of flies sterilized with gamma radiation [3] prompted worldwide studies to explore other applications for this unique method. The recent progress in radiation-sterilization research now under way at laboratories of the United States Department of Agriculture is reviewed in this paper. Limited reference is also made to information from other sources.

The most important requirements for successful application of the sterile-male release method are ability to mass-produce and sterilize the pest without serious damage to mating competitiveness or other behaviour, and sustained releases of sterile males at overflooding rates that will void the reproductive potential of the wild population [19].

2. RADIATION STERILIZATION AND INFLUENCE ON BEHAVIOUR

Three destructive tropical fruit flies, the oriental fruit fly (Dacus dorsalis Hendel), the melon fly (Dacus cucurbitae Coquillett), and the Mediterranean fruit fly (Ceratitis capitata Weidemann) have been studied intensively in Hawaii [6, 32, 33, 36]. The minimum dosage required to prevent egg-laying and render the males incapable of fertilizing females is about 10 000 r when the radiation is applied to late-stage pupae, with no significant difference among the three species. At sexual maturity the testes in treated individuals are full of sperm, but no further production of sperm occurs and few remain after 30-50 d. In cage tests with irradiated males in competition with normal males, the irradiated males were less than half as effective as normal males. In large-cage tests, however, ratios of irradiated to normal flies ranging from 7.5:1 to 20:1 suppressed reproductive potential by as much as 92% in two generations.

In studies with the Mexican fruit fly (Anastrepha ludens Loew) 12-d-old pupae were sterile after exposure to 5000 r of gamma radiation [30]. Both sexes were permanently sterilized and no eggs were produced by females. Emergence and longevity appeared to be normal. The sexual aggressiveness of young treated males was not affected. However, 30- to 39-d-old sterilized males were much less effective than normal males in competitive mating trials. More recent studies [29] indicated that, after irradiated males had mated with virgin females five or six times, few motile sperm remained in the testes and few or no sperm could be found in the spermatheca. In cage tests with ratios of sterile Mexican fruit flies to normal flies ranging from 1:1 to 50:1, fertility was greatly reduced, almost completely so at the 50:1 ratio.

In tests to determine sensitivity of the Mexican fruit fly to dose-rate of gamma radiation [30], 12-d-old pupae were irradiated at rates ranging from 10 to 90 r/min. At a dosage of 2000 r females deposited fewer eggs and males became less fertile as the dose-rate increased. No difference in emergence or longevity was observed in adults developing from pupae that were treated with 5000 r at 42.8 r/min or at 2695 r/min and there was no effect on sterility when treatment was administered in one dose of 5000 r or in two doses of 2500 r with a 24-h interval between radiation periods [29]. In sterilization of C. hominivorax it was found generally that multiple irradiation was more harmful than a single dose [11].

Mexican fruit-fly pupae have also been irradiated in atmospheres of nitrogen flowing at 3 l/min and of oxygen flowing at 5 l/min [29]. In nitrogen irradiation at 7000 r, a dosage of 2000 r higher than that required for sterilization in normal air, there was no effect on egg production or viability as had been noted in tests with C. hominivorax [20]. Females from pupae irradiated at 7000 r in oxygen laid some viable eggs, a result difficult to explain, since high oxygen tension normally increases the radiosensitivity of insect cells [10].

The influence of temperature on effects of radiation on insects has not been investigated extensively, but according to some investigators, temperature may be a critical factor [20], or at least have some significance [10]. Irradiation of dermestid larvae at 5000 rad before treatment with heat at 47°C interrupted the life-cycle, an effect that was not accomplished by either treatment alone [18].

Anopheles quadrimaculatus Say can be sterilized with 8 865 to 12 900 r of gamma radiation applied to the pupal or adult stage [9]. Irradiation of the pupae did not affect adult emergence, but there was considerable mortality of irradiated mosquitoes for the first 3 d after emergence. No gross differences were found between normal and sterile males with regard to sexual development and mating behaviour [36].

Aëdes aegypti L. may be sterilized with 11 000 to 18 000 r of gamma radiation [27]. In laboratory tests the production of viable eggs was greatly reduced when normal females were caged with normal and sterile males at a ratio of the latter of 1:20 [26].

Studies conducted on Drosophila melanogaster Meigen at Beltsville, Maryland, in 1962 indicated that untreated females mated with males exposed to 5 kr of gamma radiation in the larval and 20 kr in the pupal or adult stage deposited the normal number of eggs, none of which hatched. The longevity of males or females exposed in the pupal or adult stage was not affected by the radiation treatment, but males and females irradiated in the larval stage were shorter-lived than untreated insects. Sterile males confined with normal males and females reduced the number of progeny [15]. Immediately after treatment, 3- to 4-d-old males exposed to 16 kr did not mate as readily or as many times with virgin females as untreated males or males exposed to 8 kr. Males exposed to 16 kr recovered within 24 hours and their mating frequency and behaviour were normal [16].

Adults of the Mexican bean beetle (Epilachna varivestis Mulsant) were sterilized by exposure to 10 or 20 kr of gamma radiation. Pupae were more susceptible than adults. Dosages ranging from 1 to 16 kr resulted in sterile females, whereas males were sterilized at 4 to 16 kr. Larvae were more

susceptible to direct radiation effects than pupae and pupae were more susceptible than adults [17].

In studies with the European corn borer (Ostrinia nubilalis Hübner) at Ankeny, Iowa, untreated virgin female moths mated with male moths treated at 1 d after emergence with 32 kr of X-rays laid eggs of which only 1% hatched. Irradiated males competed equally with untreated males for females. Moths caged together at a ratio of 8 irradiated males to 4 untreated males to 8 untreated females resulted in 39.4% hatch of eggs. The survival of irradiated males compared favourably with that of untreated males under laboratory conditions. Female pupae were more susceptible to irradiation than males and younger pupae were more susceptible than older pupae [35].

At Brownsville, Texas, males of the pink bollworm (Pectinophora gossypiella Saunders) from irradiated 7-d-old pupae mated with untreated females resulted in complete sterility when irradiated at 60 kr, and at 30-60 kr no more than 1.7% of the eggs hatched. Exposure at 40 kr was sufficient to sterilize females. The longevity of males from 7-d-old pupae treated with 35-90 kr was significantly shorter than that of controls [28].

Irradiation of virgin males of the boll weevil (Anthonomus grandis Boheman) at 10 kr resulted in transient sterility, whereas 15 kr produced permanent sterility; however, these doses caused very rapid mortality of both sexes [8].

At New Haven, Connecticut, treatment of the gypsy moth (Porthetria dispar L.) with gamma radiation showed that males can be successfully sterilized and that the optimum dosage is 20 kr for 9- to 11-d-old pupae. The competitiveness and efficiency of treated males under natural conditions remain to be determined [13].

In an exploratory study with the stable fly (Stomoxys calcitrans L.) at Kerrville, Texas, 5 kr of gamma radiation to the late pupal stage resulted in complete sterility of both sexes. Although longevity was not measured, it appeared that there was very little effect from this dosage [14].

In laboratory studies conducted at Corvallis, Oregon, both sexes of the hornfly (Haematobia irritans L.) were sterilized by 5 kr of gamma radiation and longevity was unaffected. When flies from irradiated and untreated pupae were allowed to emerge together in screened cages, the eggs obtained were less viable than those in the control in every test [22].

Studies also at Corvallis, Oregon, with Culex tarsalis Colquillett showed that the male was sterilized at 15 kr and the female at 5 kr, with very little effect on longevity. Competitiveness was reported as good, but the study is still incomplete [7].

In exploratory tests conducted at Kerrville, Texas, with the Lone Star tick (Amblyomma americanum L.), females were sterilized at relatively low dosages, 500 to 2500 r. Higher dosages were required for males. Newly emerged adults were more easily sterilized than nymphs [12].

The scorpion (Centruroides limpidus Karsch) was treated with radiation in 4 lots of 15. At 4500 r there was little or no effect and one scorpion lived 105 d after treatment. At 8000 r the scorpions remained motionless and refused food; however, one lived for 33 d after treatment. At 10 000 r all were dead after 17 d. At 11 500 r all scorpions were dead at 7 d. In a control of 100 some individuals lived 6 months [24].

3. INDUCED STERILITY IN INSECT CONTROL AND ERADICATION

An economical method of rearing insects in large numbers is a requirement for utilization of the sterile-male technique [19, 36]. The availability of such methods for mass-rearing several species of tropical fruit flies made it possible to initiate field tests after appropriate radiation sterilization dosages were determined. A field test of the effect of releases of sterile Mediterranean fruit flies was undertaken in May, 1959, in an isolated 12-mile^2 host-fruit area on the island of Hawaii at an altitude of 3600-6000 ft [33]. Nearly 2 months were required for each generation of Medfly at this elevation, where temperatures seldom exceeded 60°F in the shade for more than 2-6 h/d. The test was terminated 1 July 1960, after a total of 18.7 million flies irradiated at 10 000 r in the pupal stage had been released. A mean reduction of approximately 90% in the population of Medflies was obtained.

In December 1960, releases of sterile oriental fruit flies were started on the 33-mile^2 island of Rota, an isolated island in the Western Pacific, following a year's trapping to determine the normal population density and trends. In this experiment the flies were reared to the pupal stage and irradiated in Hawaii and then shipped by air to Guam for aerial and ground releases of adults on Rota. Progress was evaluated by means of trap catches for adults and the holding of fruit samples for emergence of larval populations. The sterile flies were released weekly by air along flight lines $\frac{1}{2}$ mile apart running lengthwise to the island and from ground emergence cages placed at strategic locations [33]. This oriental fruit fly experiment was terminated in June, 1962, after 21 months, during which an estimated 410 million sterile flies were released on Rota. The target overflooding ratio of 10 to 1 of sterile to normal males was never achieved on Rota on an island-wide, sustained basis. Even though its objective was not achieved, the test nevertheless provided a wealth of valuable experience and knowledge of factors that may influence effective application of the method to an unusually abundant insect [34].

In September, 1962, releases of sterile melon flies, a much less abundant species than the oriental fruit fly on Rota, were initiated after the native population had first been reduced with poison bait sprays applied around farms and other host areas. Ratios higher than 30:1 of sterile-to-normal flies were quickly attained and complete suppression of the melon-fly population is anticipated [34]. In several recent weeks there has been no evidence of infestation in preferred hosts.

Studies involving releases of Mexican fruit flies sterilized with gamma radiation have been conducted at two locations in Mexico. In 1961, at one location, a ratio of 66:1 gravid-normal flies was achieved and the larval population in mangoes reduced to an unusually low level of 0.37 per lb. In 1962, with a high natural population, a ratio of 8:1 was insufficient to provide population suppression [29].

A. quadrimaculatus males sterilized in the pupal stage were released into natural populations of quadrimaculatus at two localities in Florida [37]. During an 11-month period 328 900 males were liberated at 9 to 10 release points on a small semi-isolated island in Lake Okeechobee, and 104 700 were liberated during an 11-wk period at two release points within an extensive

breeding area in a swamp at the south end of Lake Panasoffkee, Sumter County. It was concluded that the release of sterile males may have influenced the abundance of quadrimaculatus in the Okeechobee experiment when the natural population was in a seasonal decline, but such a release had no effect when conditions were more favourable for reproduction and development. The release of sterile males in the Lake Panasoffkee area did not conclusively demonstrate any induced sterility in wild females. Further studies on the biology and behaviour of this species are being undertaken.

A sterile release test with A. aegypti was made in two areas in the vicinity of Pensacola, Florida [27], during which 3 912 000 sterile pupae were received for liberation in 1960 (96% males) and 6 708 600 for liberation in 1961 (97% males). It was concluded from the results of the experiment that the adaptation of the sterile-male method for mosquito control will require additional biological investigations, especially of the dispersion of males under field conditions.

A co-operative programme to eradicate the screw-worm throughout the south-eastern United States by means of releases of sterile flies was undertaken in 1958 by the United States Department of Agriculture and the State of Florida [23, 36]. An average of 50 million screw-worm flies were reared, sterilized and released per week to achieve eradication in less than 2 yr in the southern United States east of the Mississippi River.

Early in 1962, a co-operative programme was undertaken by the United States Department of Agriculture, the Southwest Animal Health Research Foundation, the Texas Animal Health Commission and other public and private groups in several States to utilize releases of sterile flies to eradicate the screw-worm from the south-western United States and to maintain a barrier against re-infestation from Mexico. The rate of sterile fly releases in the areas in Texas where the insect is normally able to overwinter had reached over 113 million/wk by early February 1963.

Inability to identify released insects positively is a serious handicap in evaluation. A genetic strain of white-marked oriental fruit flies which had been found to occur naturally in 0.5% of the wild Rota population was developed in Hawaii. In comparative tests, the white-marked strain has appeared to be equal in behaviour and reproductive capacity and compatible with normal yellow laboratory flies. The white-marked oriental fruit fly strain was used in the Rota test beginning with the releases made in August 1961 [33]. Mexican fruit-fly rearing stocks are observed for easily detectable aberrations which might be developed as genetic markers and F_1 and F_2 progeny or males irradiated with 1500 r and mated with untreated females are scanned. A female with a dark V on the scutellum was obtained from a total of 30 000 F_2 -generation flies, but death occurred before sexual maturity [29].

In the search for mutants of the screw-worm useful for marker stocks, the flies scanned are from three sources, untreated laboratory-reared flies, progeny from irradiated normal parents and progeny from irradiated flies inbred for two generations. A number of mutants have been observed but none has been fully compatible and competitive with wild flies [21].

4. IONIZING RADIATION AS A COMMODITY TREATMENT

The development of irradiation methods for treatment of fruit and other commodities susceptible to infestation by fruit flies and other insects may offer a means of quarantine treatment that is more effective and less undesirable than fumigation or other treatments.

The first successful test with ionizing radiation to free a commodity from insects apparently was with tobacco infested with the tobacco or cigarette beetle (Lasioderma serricorne F.) in 1916 [31]. In the Medfly eradication campaign in Florida in 1929, a few exposed larvae and larvae within fruit were treated with X-rays. It was concluded that the exposures were insufficient to kill larvae, either exposed or within fruit [25].

Studies in Honolulu, Hawaii, indicated that 300 kr was not immediately lethal to the immature stages of the oriental fruit fly. However, the development of irradiated eggs and larvae to the adult stage was prevented by dosages of 7.5 and 15 kr [1]. The results of further investigations with the immature stages of the oriental fruit fly, the melon fly and the Medfly suggested that comparatively low dosages in the range 15 - 20 kr would provide an effective commodity treatment for fruit-fly-infested fresh fruits and vegetables [2].

Larvae of the mango weevil (Sternochetus mangiferae F.) in mango seeds treated with 5 kr of gamma radiation failed to develop to adults and were prevented from forming pupae by an exposure of 12.5 kr. Sexually mature adults irradiated in shell vials were sterilized by 5 kr and sexually immature adults treated within the bare mango seed were sterilized by 15 kr [2].

Grapefruit infested with eggs and first-stage larvae of the Mexican fruit fly exposed to 5 krad showed no insects or insect damage when dissected. Fruit infested with mature larvae produced numerous pupae; however, no adult flies emerged from pupae from fruit treated at 5 krad or higher [5].

Mangoes infested with the Mexican fruit fly were treated with gamma radiation at dosages ranging from 500 to 5000 r at the rate of 1500 r/min. Flies surviving the 1000-r dose did not oviposit and only one malformed male survived at each of the 2000 and 2500-r treatments and no adults emerged at 3000 r or above. In further studies, X-ray radiation appeared to be as lethal to the Mexican fruit fly as gamma radiation [4].

REFERENCES

[1] BALOCK, J.W., CHRISTENSON, L.D. and BURR, G.O., Proc. Hawaii. Acad. Sci. (1956) 18.
[2] BALOCK, J.W., SEO, S.T. and FUKOMOTO, M., J. Econ. Entomol. (in press).
[3] BAUMHOVER, A.H., GRAHAM, A.J., BITTER, B.A., HOPKINS, D.E., NEW, W.D., DUDLEY, F.H. and BUSHLAND, R.C., J. Econ. Entomol. 48 4 (1955) 462-8.
[4] BENSCHOTER, C.A. and TELICH, C., J., Unpublished Report, U.S.D.A., A.R.S., Ent. Res. Div., Mexico City, Mexico (1963).
[5] BROWNELL, L.E. and YUDELOVITCH, M., Radioisotopes and Radiation in Entomology, IAEA, Vienna, (1962) 193-206.
[6] CHRISTENSON, L.D., 10th Internatl. Congr. Ent. 3 (1956) 11-18.
[7] DARROW, D.I. and EDDY, G.W., Unpublished Report, U.S.D.A., A.R.S., Ent. Res. Div. (1963).
[8] DAVICH, T.B. and LINDQUIST, D.A., J. Econ. Entomol. 55 (2) (1962) 164-7.

[9] DAVIS, A. N., GAHAN, J. B., WEIDHASS, D. E. and SMITH, C. N., J. Econ. Entomol. 52 (5) (1959) 868-70.
[10] DESROSIER, N. W. and ROSENSTOCK, H. M., "Radiation Technology in Food and Agriculture", Avi. (1960) 395.
[11] DIXON, E., J. Econ. Entomol. 55 (1962) 826-7.
[12] DRUMMOND, R. O., Unpublished Report, U. S. D. A., A. R. S., Ent. Res. Div., Kerrville, Texas (1962).
[13] GODWIN, P. A., RULE, H. D. and WATERS, W. E., Unpublished Report, U. S. D. A., Forest Service, New Haven, Connecticut (1961).
[14] HARRIS, R. L., Unpublished Report, U. S. D. A., A. R. S., Ent. Res. Div., Kerrville, Texas (1961).
[15] HENNEBERRY, T. J., J. Econ. Entomol. (in press).
[16] HENNEBERRY, T. J. and McGOVERN, W. L., J. Econ. Entomol. (in press).
[17] HENNEBERRY, T. J., SMITH, F. F. and McGOVERN, W. L., J. Econ. Entomol. (in press).
[18] HORNE, T. and BROWNELL, L. E., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 233-54.
[19] KNIPLING, E. F., J. Econ. Entomol. 48 4 (1955) 459-62.
[20] LaCHANCE, L. E., Proc. N. Cent. Br. Ent. Soc. Amer. 17 (1962).
[21] LaCHANCE, L. E. and HOPKINS, D. E., J. Econ. Entomol 55 (5) (1962) 733-7.
[22] LEWIS, L. F. and EDDY, G. W., Unpublished manuscript, U. S. D. A., A. R. S., Ent. Res. Div., Corvallis, Oregon (1963).
[23] LINDQUIST, A. W., Pest Control 29 (6) (1961).
[24] MAZZOTTI, L., RHODE, R. H., LOPEZ, D., F. and TELICH, C., J., Rev. Inst. Salubr. Enfer. Trop. (Mex.) XXI (1961) 125-7.
[25] McBRIDE, O. C., Unpublished Report, U. S. D. A., Bur. Ent., Orlando, Florida (1959).
[26] McCRAY, E. M., Jr., JENSEN, J. A. and SCHOOF, H. F., Proc. N. J. Mosq. Ext. Assn. 48 Ann. Mtg. (1961) 110-5.
[27] MORLAN, H. B., McCRAY, E. M., Jr. and KILPATRICK, J. W., Mosquito News 22 3 (1962) 295-300.
[28] OUYE, M. T. and GARCIA, R. S., Unpublished Report, U. S. D. A., A. R. S., Ent. Res. Div., Brownsville, Texas (1962).
[29] RHODE, R. H., Unpublished Reports, U. S. D. A., A. R. S., Ent. Res. Div., Mexico City, Mexico (1963).
[30] RHODE, R. H., LOPEZ, D., F., EGUISA, F. and TELICH, C., J., J. Econ. Entomol. 54 (1962) 202-3.
[31] RUNNER, G. A., J. agric. Research 6 (1916) 383-8.
[32] STEINER, L. F. and CHRISTENSON, L. D., Proc. Hawaii. Acad. Sci. 1955-1956 (1956).
[33] STEINER, L. F., MITCHELL, W. C. and BAUMHOVER, A. H., Internat. J. appl. Radiation and Isotopes (1962) 115-22.
[34] STEINER, L. F., Unpublished Reports, U. S. D. A., A. R. S., Ent. Res. Div., Honolulu, Hawaii (1962-3).
[35] WALKER, John R. and BRINDLEY, T. A., Unpublished Report, U. S. D. A., A. R. S., Ent. Res. Div., Ankeney, Iowa (1962).
[36] WEIDHAAS, D. E., SCHMIDT, C. H. and CHAMBERLAIN, W. F., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 257-65.
[37] WEIDHAAS, D. E., SCHMIDT, C. H. and SEABROOK, E. L., Mosquito News 22 3 (1962) 283-91.

DISCUSSION

M. S. QURAISHI: How were the male mosquitoes separated from the female mosquitoes for the release of males?

W. E. STONE: I regret I cannot tell you that because I am simply quoting what was done by Dr. Schmidt and Dr. Morlan and their associates. Dr. Schmidt could undoubtedly tell you.

M. S. QURAISHI: We have found that the mechanical separator described by McCray and Morlan is quite efficient in separating male and female larvae of Anopheles stephensi, but of course you do not get 100% separation. Perhaps Dr. Schmidt could throw some more light on this.

C.H. SCHMIDT: In the experiments on the release of sterile males for the control of Anopheles quadrimaculatus (ref. [37] to the paper), the sex separation was carried out in the adult stage. Within 24-48 h after the adults emerged, they were inactivated in a cold room (35°-40°F), the females removed and the males transferred to cages for release in the test area. Females were not released, since their bites would have caused annoyance and their presence in the resting stations in the test area would have introduced errors in the weekly adult counts. Before we undertake a large-scale test we shall have to devise some better means of separating the sexes. This was much too tedious.

J.W. MILES: Dr. Schmidt, when you irradiated these insects, the irradiation was done on the pupae, was it not, the adults being separated afterwards? In previous work, by McCray et al. (ref. [26] to the paper), I believe they irradiated the male pupae after separation.

C.H. SCHMIDT: Yes, we irradiated quadrimaculatus pupae which were not more than 24-h old.

P. NARDON: You said, Dr. Stone, that you cultured the Mexican fruit fly in order to see whether any aberrations appeared. Personally, I think aberrations are bound to appear. I have observed them with Sitophilus grain weevils, and the paper I am to present tomorrow * has some bearing on this matter. But could you, in particular, give us some indication as to the likelihood of reduced fertility? In my opinion it is particularly on the physiological side that one may expect important changes.

W.E. STONE: The Mexican fruit flies were exposed to 1500 r, and this was sufficient to reduce fertility somewhat; we believe, however, that fertility can be restored if a satisfactory mutant is obtained. As far as other aberrations are concerned all I can say is that out of some 20 000 irradiated flies we found an inverted "V" on the scutellum in one female, but unfortunately she died before she could lay eggs. That often happens, the geneticists tell me, in working with stocks that have been irradiated.

A. SÜSS: Have you found any relation between temperature and sterilization dose?

W.E. STONE: All our sterilization has been done at about 25-27°C — more or less at room temperature. We have not, ourselves, investigated the effect of temperature in relation to irradiation, but work done by Dr. Nair on this question was referred to by Horne and Brownell in the paper cited as ref. [18] to my paper. The paper by Nair and Rahalkar on their work with the Khapra beetle † contains further interesting information on this subject. I think that D.J. Jeffreys has also done some work on the relation of temperature to irradiation in the case of Sitophilus granarius.

R. von BORSTEL: I think the United States Department of Agriculture is to be congratulated on the marvellous work done on eradication of insect pests by the male irradiation method. I would like to know why the particular dose of 1500 r was selected for the screening of mutations in Anastrepha sperm.

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* LAVIOLETTE, P. et NARDON, P., Influence de l'irradiation sur les adultes de Sitophilus sasakii takahashi (Curculionidae) et leurs descendants, these Proceedings.

† NAIR, K.K. and RAHALKAR, G.W., Studies on the effects of gamma radiation on the different development stages of the Khapra beetle, Trogoderma granarium Everts., these Proceedings.

W.E. STONE: The 1500-r exposure was selected for Anastrepha ludens arbitrarily, as it is between 1000 r, which has little effect on oviposition and hatch, and 2000 r, which is considered a critical dosage.

R. von BORSTEL: Who is carrying on that work?

W.E. STONE: LaChance. A very full account of it appears in the article cited as ref. [21] to my paper.

M. FRIED: Have you any more recent information as to the results of the melon-fly tests on the island of Rota?

W.E. STONE: Nothing more than what is said in the paper. I would like to mention that the main problem in all our eradication work by the sterile-male technique is the problem of ensuring sufficient isolation. You may think you have pretty good isolation but then the insects begin to come in: Steiner and his associates in Hawaii have found on the island of Rota marked melon flies that were released on the island of Guam 40 miles away; in Mexico, we are facing a similar situation with the Mexican fruit fly. You would think that in a country as large as Mexico we would be able to find isolated places to carry out our experiments, but it is almost impossible. Now, Greece, I should say, would be situated ideally, with its many islands, some of them quite far apart. This problem of isolation is a tremendous problem: in the south-western United States, for a week or two, there were no screw-worm cases, and then all of a sudden they began to reappear.

M.S. QURAISHI: Talking of sterilizing insects, I was wondering if Dr. Stone, or any other scientist present, would like to comment on the chemical sterilants regarding which quite a few papers have appeared in recent literature.

W.E. STONE: In Mexico, most of our sterile release work at the present time is being done with flies sterilized in the pupal stage with TEPA. We have a paper in press at the present time, which should appear in six to eight months in the Journal of Economic Entomology, reporting on the results of release of flies sterilized with TEPA in a ten-acre mango grove in Mexico. If we had had complete isolation I think our results would have been very much better, but they were so good that for the first time in history at that particular grove about 90% of the crop was harvested as acceptable fruit; in the control area, about a mile away, all the fruit was gone two or three weeks previously. This product TEPA, which we selected out of some 1500 for development at the present time, is a most promising material, though it should be handled with care and we make sure that our workers use gloves, and when the pupae are dipped we have good extractor fans operating in the room. We also have chemosterilant stations where we are testing, but we now have them under guard night and day.

K. van ASPEREN: Do you know of any experiments on the development of resistance to ionizing radiation in insects?

W.E. STONE: No, I do not. There are workers here much more capable of answering that question than I.

R. von BORSTEL: I know of only one large-scale experiment in insects designed to select for mutations conferring resistance to radiation. This was an experiment carried out by Bruce Wallace on populations of Drosophila melanogaster under chronic radiation conditions at such a high level that population decline was continuous. Every few generations it was necessary

to halt the irradiation, build up the population, and begin the irradiation again. Radiation-resistant flies were not obtained.

R. DELATTRE: The insects mentioned in your paper, Dr. Stone, are all of the holometabolous type: dipterous, lepidopterous, etc. Is there any inherent difficulty in applying this method to hemimetabolous insects?

W.E. STONE: I have never been associated with any studies in which hemimetabolous insects have been irradiated. The only cases I know of are the Lone Star tick, which is not strictly speaking an insect, and the scorpion in Mexico. I am sure that other work has been done, but I do not know who has done it.

H. HUQUE: Do you know whether any irradiation work is being done by the United States Department of Agriculture on plant quarantine?

W.E. STONE: I do not know of any such work in the United States as such, within the continental limits. In the work we did for the United States Department of Agriculture in Mexico with the Mexican fruit fly and the work I referred to with the Dacus, the Mediterranean fruit fly and the mango weevil, all of which was done in Hawaii, the idea of this work was in fact to develop a possible quarantine method. But I cannot tell you what, if anything, has been done in the United States itself.

ERADICATION OF WHITE GRUB (MELOLONTHA VULGARIS F.) BY THE STERILE - MALE TECHNIQUE

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Abstract — Résumé — Аннотация — Resumen

ERADICATION OF WHITE GRUB (MELOLONTHA VULGARIS F.) BY THE STERILE-MALE TECHNIQUE. Laboratory tests indicated that an X-ray dose of 3000 r was sufficient to induce sterility in male cockchafers. During two flight periods, sterilized males were released among a natural population in order to eliminate white grubs in a general farming region of north-western Switzerland. In 1950 an outbreak of this pest was reduced by a chemical treatment. Gradation had been watched during every flight from 1953 to 1962.

In 1959 five areas, each with a surface of about 30 ha, were selected to serve as:

(a) The treated area, where the males were captured, irradiated and released;

(b) The "bank," where cockchafers were collected and the males were irradiated for release in area a; and

(c) Control areas, where undisturbed gradation was observed.

The males were irradiated in a therapeutical X-ray unit. Irradiated males were hand-painted in order to estimate the ratio of sterilized males by means of the isotopic dilution technique.

In 1959, for the first treatment, about 6 l of sterilized males, representing about 50% of the male population, were released in (a).The white grub infestation sampled in grassland dropped thereafter to about two-thirds of that in the other areas. The reproduction rate was less than unity only in (a). A further reduction of the population in (a) to one-tenth of that in (b) and (c) was observed when the number of surviving cockchafers was estimated in 1962. The greatest mortality from 1959 to 1962 occurred in (a).

In 1962, for the second treatment, a total of 17 l of irradiated males was released in (a). At least 76% of the male population of (a) had been sterilized. The following sampling of the white grub population showed complete eradication in (a). Some reduction was also observed in (b) and (c) due to drought in the whole region.

It has been demonstrated that the sterile-male technique may successfully be applied to an insect pest in an area which is not strictly isolated geographically, the females of which mate several times and the breeding of which in large masses is not feasible because of the long breeding cycle.

ÉRADICATION DU VER BLANC (MELOLONTHA VULGARIS F.) PAR LA MÉTHODE DU LÂCHER DE MÂLES STÉRILES. Des essais en laboratoire ont montré qu'une dose de rayons X de 3000 r suffit pour stériliser les hannetons mâles. Des mâles stérilisés ont été lâchés, au cours de deux apparitions, dans une population naturelle en vue d'éliminer les vers blancs dans une région essentiellement agricole du nord-ouest de la Suisse. En 1950, une invasion de hannetons avait pu être enrayée à l'aide d'un traitement chimique. L'évolution ultérieure a été observée à chaque apparition, de 1953 à 1962.

En 1959, on a choisi cinq zones d'environ 30 hectares chacune, réparties en trois catégories:

a) Zone traitée, où les mâles étaient capturés, irradiés et relâchés;

b) Zone de réserve, où les hannetons étaient ramassés, les mâles irradiés et relâchés en a;

c) Zones témoins, où l'on observait l'évolution naturelle.

L'irradiation des mâles était effectuée dans un appareil de roentgenthérapie. Les mâles irradiés recevaient des marques peintes à la main, afin de pouvoir évaluer la proportion de mâles stériles par la méthode de dilution isotopique.

En 1959, année du premier traitement, on a relâché en a) environ 6 l de mâles stérilisés, ce qui représentait près de 50% de la population mâle. D'après les échantillons prélevés dans les prés, le nombre de vers blancs n'était plus par la suite que les deux tiers de leur nombre dans les autres zones. Le taux de reproduction n'était inférieur à l'unité que dans la zone a). En évaluant le nombre des hannetons survivants en 1962, on a constaté que la population de a) était tombée à un dixième de celle de b) et c). C'est en a) que la mortalité a été la plus élevée entre 1959 et 1962.

En 1962, année du deuxième traitement, on a relâché en a) 17 l de mâles irradiés. La population mâle de a) avait été stérilisée dans la proportion d'au moins 76%. Les échantillons prélevés par la suite ont montré que l'éradication des vers blancs en a) était absolue. On a également observé une certaine diminution des larves en b) et c), due à la sécheresse qui régnait dans toute la région.

Il a été démontré que la méthode du lâcher de mâles stériles peut être appliquée avec succès dans la lutte contre un insecte nuisible qui se trouve dans une région qui n'est pas strictement isolée du point de vue géographique, dont les femelles s'apparient plusieurs fois et dont l'élevage en masse est impossible en raison de la durée du cycle évolutif.

УНИЧТОЖЕНИЕ ЛИЧИНОК МАЙСКОГО ЖУКА (MELOLONTHA VULGARIS F.) МЕТОДОМ СТЕРИЛИЗАЦИИ МУЖСКИХ ОСОБЕЙ. Лабораторные испытания показывают, что доза 3000 р достаточна для стерилизации мужских особей майского жука. В течение двух вылетов стерилизованные самцы выпускались на волю среди необлученных популяций для ликвидации хрущей в районе с неспециализированным сельским хозяйством, расположенном в северо-западной Швейцарии. В 1950 году широкое распространение этих вредителей было предотвращено применением химикалий. Спаривания наблюдались при вылете с 1953 по 1962 год.

В 1959 году были отведены пять участков площадью 30 га, на которых создали:

А - экспериментальный участок, на котором мужские особи вылавливались, облучались и выпускались снова на волю;

Б - участок для разведения, где майские жуки собирались, их мужские особи облучались и выпускались на участок А;

В - контрольные участки, где наблюдались спаривания без внешнего вмешательства.

Самцы облучались на обычной терапевтической рентгеновской установке. Облученные самцы окрашивались вручную для определения пропорции стерилизованных самцов методом изотопного разведения.

В 1959 году в качестве первого опыта на участок А было выпущено около 6 л стерилизованных самцов, что составляло около 50% всей популяции самцов. Пробы, взятые с пастбищ, показали, что заражение хрущами сократилось приблизительно на 1/3 по сравнению с другими участками. Коэффициент размножения был меньше единицы только на участке А. Когда в 1962 году была произведена оценка числа выживших майских жуков, то на участке А было обнаружено дальнейшее сокращение популяции на 1/10 по сравнению с участками Б и В. Наибольшая смертность с 1959 до 1962 года наблюдалась на участке А.

В 1962 году для второго опыта на участке А было выпущено 17 л облученных самцов. По меньшей мере, 76% популяции самцов на участке А было стерилизовано. Последующее определение популяции хрущей показало их полное истребление на участке А. Некоторое сокращение наблюдалось также на участках Б и В в результате засухи в этом районе.

Было показано, что метод стерилизации самцов может с успехом применяться на участке без строгой географической изоляции для борьбы с насекомыми-вредителями, самки которых спариваются по нескольку раз в сезон и разведение которых в больших количествах неосуществимо ввиду значительной продолжительности цикла их выведения.

ERRADICACIÓN DE LA LARVA DEL ABEJORRO (MELOLONTHA VULGARIS F.) POR LA TÉCNICA DE LOS MACHOS ESTERILIZADOS. Ensayos realizados en laboratorio indicaron que para esterilizar a los abejorros machos basta con una dosis de rayos X de 3000 roentgens. Durante dos períodos de vuelo se soltaron machos esterilizados en una región agrícola del noroeste de Suiza con objeto de erradicar las larvas. En 1950 esta plaga había sido combatida con procedimientos químicos. Entre 1953 y 1962 se observó la actividad en todos los vuelos.

En 1959 se seleccionaron cinco zonas de 30 hectáreas cada una que sirvieron de:

a) Zona de tratamiento, en la que se capturaron, irradiaron y soltaron machos;

b) Zona de reserva, en la que se recogieron abejorros y se irradiaron machos que se soltaron en a);

c) Zonas de control, en las que se observó la actividad normal.

Los machos fueron irradiados con un aparato terapéutico de rayos X. Después de la irradiación se les puso una marca de color para poder calcular su porcentaje mediante la técnica de dilución isotópica.

En 1959, durante el primer tratamiento, se soltaron en la zona a unos 6 l de machos esterilizados, lo que representaba aproximadamente el 50% del total de machos de dicha zona. La cantidad de larvas recogidas en los prados después del tratamiento representó unos 2/3 de la correspondiente a las zonas de control. La razón de reproducción sólo fue inferior a la unidad en la zona a). Cuando se calculó, en 1962, el número de

abejorros supervivientes se observó que en la zona a había disminuido hasta 1/10 en comparación con las zonas b) y c). De 1959 a 1962 la cifra más elevada de mortalidad se observó en la zona a).

En 1962 se procedió en esta zona a un segundo tratamiento para el que se soltaron 17 1 de machos irradiados. Se había esterilizado el 76% por lo menos de los machos. El nuevo muestreo de larvas demostró que en la zona a) el exterminio había sido completo. Debido a la sequía que reinó en toda la región se observaron también disminuciones en las zonas b) y c).

Se ha demostrado que la técnica de los machos esterilizados puede aplicarse con éxito a insectos de una región que no esté estrictamente aislada desde el punto de vista geográfico, cuyas hembras copulen varias veces, y que no sea posible criar en grandes cantidades debido a la larga duración de su ciclo de reproducción.

0 INTRODUCTION

The literature of genetics and cytology dealing with the effects of radiation on insects have been reviewed by MULLER (1940, 1941) [16,17], LEA (1947) [14] and CATCHESIDE (1948) [4]. Valuable reviews of the literature on the action of ionizing radiation on insects, each including more than 200 references, have been given by HILCHEY (1957) [6] and GROSCH (1962) [5]. According to the findings reported, X-rays and gamma-rays cause similar effects. The extent of the changes in the germ cell depends on the dosage. Extreme doses cause the cell to degenerate. Less extensive changes may not prevent the sperm from fertilizing an egg, but the zygote usually dies in the embryonic stage. Mutations that prevent the survival of the fertilized egg are dominant lethal mutations. While the males irradiated at dosages sufficient to induce dominant lethal mutations in all the germ cells are for practical purposes sterile, since their progeny dies as embryos, they are not, technically speaking, truly sterile, because they are still capable of producing sperm which fertilizes eggs.

Of greatest economic importance is the extensive work done on the screw-worm, Callitroga hominivorax, by BUSHLAND et al. (1951, 1953) [2,3], BAUMHOVER (1955) [1], KNIPLING (1955) [12], and LINDQUIST (1955) [15]. Most of the extensive irradiation research on Coleoptera concerns pests of stored products. Only a few pests of field crops, e.g. bollworms, weevils, cane and corn borers, have been considered so far [13].

01 Biology of the cockchafer (Melolontha vulgaris F.)

In the regions concerned, most individuals of a natural population require three years for their development from egg to adult. The flight period of the adult cockchafer is concentrated in a few weeks every three years. During their flight, which usually starts about 20 April and continues through May and June, the beetles gather along the edges of or inside woods. Trees with deciduous foliage, such as oak and beech, are preferred host plants. After feeding for a short preoviposition period and mating, the females return to the adjacent fields. Oviposition occurs preferably in grassland. The chief crop losses have to be expected in the first and sometimes in the second year after the flight period. White grub damage mainly affects root vegetables, such as potatoes and sugar beet, strawberries and nurseries. Metamorphosis is completed in the summer of the second year after flight. The beetles hibernate in the soil near their exuviae at a depth of 20 - 30 cm. In the spring of the third year after the flight period, the three-year cycle

starts again with oviposition. The appearance of the first beetles may be predicted precisely by a calculation based on air temperature. The daily averages above + 8°C, beginning from 1 March, are added and when the sum of 256 ± 16°C is reached, the first mass flights can be expected (HORBER, 1955) [8]. A special behavioural feature favourable to the application of the sterile-male technique is the appearance of males in large numbers in advance of the females. A natural separation already takes place in the soil, most likely as the result of a differential threshold of sensitivity to soil temperature as between the two sexes (HORBER, 1955) [8].
females, sometimes in proportions up to 40 : 1, in catches in light traps operated during the first hour after sunset (Table I). This artificial procedure superimposed on the natural separation yields a predominantly male fraction that consequently may be sterilized and released before the appearance of the females.

On the other hand, it has to be taken into account that each female is likely to mate several times. It has not, however, been proved that several matings are necessary or of biological significance in increasing reproduction.

TABLE I

SEX RATIO OF COCKCHAFERS OBSERVED IN A LIGHT TRAP

1959 VENDLINCOURT				1962 'LES TAYES'			
Evening operated	Cockchafers trapped		Ratio M/F	Evening operated	Cockchafers trapped		Ratio M/F
	Males	Females			Males	Females	
6 May	10	35	0.3	3 May	71	26	2.7
7	80	45	1.8	7	116	35	3.3
8	124	31	4.0	12	143	142	1.0
9	44	1	44.0	16	719	210	3.7
10	43	7	6.1	21	294	205	1.4
13	2	0	∞	22	65	85	0.7
Total	303	119	2.5	Total	1408	703	2.0

02 Conventional methods of controlling cockchafer and white grub

Both chemical and mechanical means of controlling this pest, in either the larval or the adult stage, are available. Several cultural methods of preventing white grub damage are recommended (HORBER, 1954, 1958, 1961) [7,9,11]. In a number of Swiss cantons the collection of May beetles had been declared obligatory and has been carried out in some places for a century or even longer. Since 1948, several attempts have been made on a large scale to eradicate the cockchafers by treating the woods and also dispersed trees preferred as food with insecticides.Compounds based on lindane proved to be the most effective and were therefore widely used. Insecticidal treatments on a large scale during a short flight period require the avail-

ability of a considerable number of atomizers or, in difficult country, of aircraft. Very close co-operation among a well-trained technical and biological staff is necessary.

1 LABORATORY EXPERIMENTS ON THE NECESSARY X-RAY DOSAGE AND IRRADIATION EFFECTS

11 Material and methods

From 1955 to 1958 samples of cockchafers were taken in three different regions of Switzerland. They were obtained in March and April by digging them out of their hibernation sites. They were kept at temperatures below + 6°C. No food was provided before treatment. The irradiation was performed by the staff of the radiobiological laboratory of the Zürich Cantonal Hospital. The X-ray apparatus used was a Siemens "Stabilivolt" operated at 180 kV and 6 mA. During the exposure the beetles were confined to a plastic phantom in lots of about 30 to 60. Before and after treatment they were kept in lots of about 20 in small tin or aluminium cans refrigerated at 4-6°C. The boxes were filled with wet sawdust. They served equally well as transport containers.

In order to measure fertility the irradiated males were exposed in cages along with an aliquot of unsterilized females. The fecundity of females after mating was measured by counting the eggs laid into the soil layer exposed on the bottom of the cages. Fertility was recorded by exposing equal batches of 20 - 25 eggs in wet soil or vermiculite in Petri dishes at 20°C. The young white grubs were counted and removed at regular intervals.

12 Results

The X-ray dosages administered are tabulated against fecundity and fertility (Table II). It was established from these counts by interpolation that 3000 r would be the minimum dose required to obtain at least temporary sterilization. Since mortality and behaviour during copulation were not appreciably affected up to a dosage of 20 000 r, it was assumed that it would be safe to administer a dosage within the range 3000 - 5000 r.

2 APPLICATION OF THE STERILE MALE TECHNIQUE TO A NATURAL POPULATION

21 Material and methods

211 Selection of the sites serving as treated, control and "bank" areas

An opportunity to apply the sterile-male technique to a natural population of the cockchafer was recognized in the Ajoie (Canton of Berne) where gradation had been observed carefully since 1953 in an area measuring about 2 500 ha. In 1950, an insecticidal treatment had been performed in the same district in order to control an outbreak of this pest. In the subsequent flight periods a gradual regression has been observed (Table III, Figs. 1 and 2).

Five different areas were selected, each containing about 30 ha of arable land, with 13 - 15 ha of grassland and other field crops and adjacent to woods. Each area was selected to be at least 3 - 5 km from the next in order to minimize the possibility of migrations. The main criterion for

TABLE II

EFFECT OF IRRADIATION ON REPRODUCTION OF THE COMMON COCKCHAFER

Year	X-ray dose applied (r)	Number of irradiated males	Number of eggs laid by an aliquot number of non-irradiated females	Eggs hatched (%)
1955	0	27	83	56
	5 000	27	66	0
	10 000	27	84	0
	20 000	27	84	0
1956	0	30	280	10.7
	1 000	29	160	1.9
	2 000	28	126	0
	4 000	29	95	0
	8 000	29	76	0
1957	0	64	307	45
	2 000	60	637	8.7
1958	0	65	205	56.6
	2 000	63	263	5.3
	4 000	62	94	0

TABLE III

GRADATION OF COCKCHAFER AND WHITE GRUB POPULATION IN THE AJOIE FROM 1950 TO 1962
(Average number per m² in the whole region)

Year	1950	1953	1956	1959	1962
Cockchafers	-	4.9	2.05	0.69	0.91
White Grubs	59.6*	15.8	13.96	1.50	0.48

* SCHENKER [18]

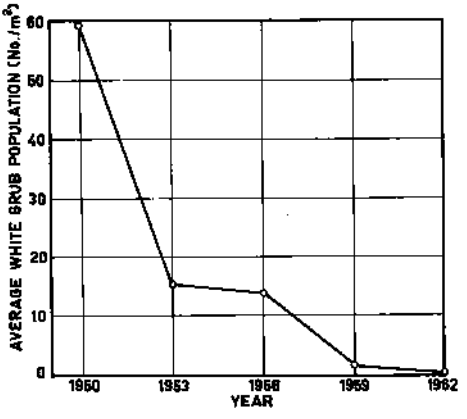

Fig. 1

Gradation of white grub population 1950 to 1962

Average number of white grubs per m^2 based on 350 sampling units in 1950, 496 in 1953, 1120 in 1956, 800 in 1959 and 864 in 1962.

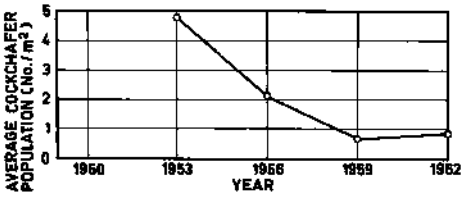

Fig. 2

Gradation of cockchafer population 1953 to 1962

Average number of cockchafers per m^2 based on 928 sampling units in 1953, 864 in 1956, 800 in 1959 and 688 in 1962.

selecting these areas for the field trial was a moderate population density both in the area to be treated and in the untreated or control areas, with a higher density for the area selected as the "bank". In the description below these areas carry the following designations (see Fig. 3):

Area A, the treated area, where males were captured, irradiated and released (Vendlincourt);

Area B, the "bank", where cockchafers were collected and the males irradiated for release in area A (Les Tayes);

Area C, the control area, where undisturbed gradation was observed (Alle = C_1, Coeuve = C_2, Lugnez = C_3).

212 Description of the areas selected for the field experiments 1959-62

The rural district surveyed since summer 1950 is situated on the northwestern foothills of the Bernese Jura ranges. The terrain stretches over a rolling landscape underlaid by Jurassic limestone and is irregularly dotted

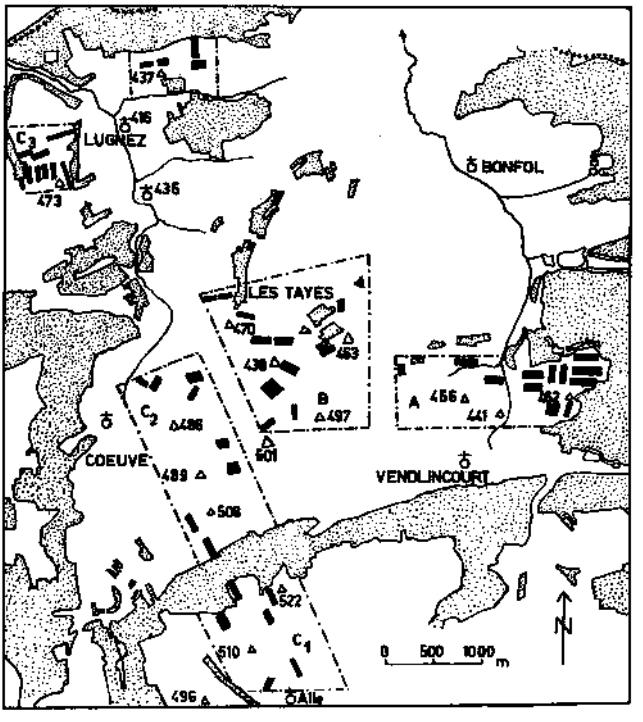

Fig.3

Field Experiment 1858 to 1962

Boundary lines -.-.- of areas A (sterilized males released, B(bank), C1-2(controls), with positions of grassland plots indicated, in each of which 16 sampling units of 0.25 m² were dug in order to estimate white grub and cockchafer populations. Small numerals indicate altitude (m above sea level).

with dolines, marked with oaks, beeches and cherry-trees. The altitude ranges from 420 to 520 m above sea-level. The villages are situated at the bottom of the valleys. The tops of the hills and some of the steeper slopes are wooded. The whole region is surrounded by woodland. Intensive farming is confined to the bottoms of the valleys, while in the higher grassland and pasture areas the arable land is predominantly planted to wheat, oats and barley. The soil consists of renzina, loam and sandy loam. Horse-breeding has been a traditional activity in this district.

The treated area A is situated near Vendlincourt. On three sides it is surrounded by woodland while to the west it is separated from the neighbouring farmland by a belt of poorly drained grassland. The enclosed area, measuring about 30 ha of open land with 14 ha of grassland, is marked out from the other areas by its lighter sandy-loam soil. During our first survey in 1953, we noticed that the mortality of the cockchafers between oviposition and flight was lowest in that area (Table IV). Farmers in that region

TABLE IV

MORTALITY CALCULATED BY COMPARING COCKCHAFER POPULATION WITH WHITE GRUB POPULATION AFTER THE PREVIOUS FLIGHT PERIOD

Area	Period of observation		
	1953 - 56	1956 - 1959	1959 - 62
A	78.9	87.6	68.8
B	84.1	91.2	63.1
C	89.7	95.8	43.8

observed that this soil was also a preferred hibernation site for the Colorado potato beetle (Leptinotarsa decemlineata Say). We therefore surmised that this area might possibly serve as an infestation centre from where outbreaks of the cockchafer may spread over the surrounding areas.

The adults emerging from the grassland in this area collected along the border of a triangular wood. One corner of this border is raised on the rim of a hill and protrudes towards the open area; it is therefore highly attractive to the emerging beetles. The wood consists of oak, beech, cherry-trees and poplar, which are preferred food trees.

The "bank" area B has a unique topographical situation in that it consists of a group of small woods called "Les Tayes". Situated on top of a hill, they attract the cockchafers from all directions over a great distance. Although the soil of the surrounding fields consists of renzina, which is not good breeding ground for white grub, cockchafers emerging from several 100 ha of the adjacent slopes are attracted to these woods. As these contain preferred food trees, they act as bait and consequently great masses of beetles may be collected along their borders.

The control areas C: Three areas situated near Alle, Coeuve and Lugnez respectively were selected because of the similarity of their topographical situation and the renzina soil they have in common. The fields and grassland from which the beetles emerge are situated on slopes topped with woodland. Near Alle the slope is exposed to the south, near Coeuve it is situated to the west, while near Lugnez a small wood stretched out along the top of a ridge attracts beetles from both hillsides. In many fields of these areas the underlying limestone of the Jura formation is barely covered by a thin renzina layer and the are therefore exposed to drought conditions sooner than the areas A and B.

Sectors of about 30 ha of cultivated surface together with the adjacent wood in each of these three districts were considered as control areas, where the undisturbed development of the population was observed from 1953 to 1962.

213 Estimation of the cockchafer and white grub populations

Several weeks before the flight started the population density was estimated by digging and searching through about 800 sampling units each consisting of 0.25 m^2 of surface area down to a depth of 20 - 40 cm. Between 112 and 256 such sampling units were dug in each of the five areas, depending on their size. The samples were grouped and classified according to the distribution of grassland in the area. In each area 7 - 16 plots of grassland or fields of about 0.4 - 0.6 ha surface were sampled. In each plot 16 random 0.25-m^2 sampling units were averaged. The number and sex of beetles were recorded separately for each plot.

In 1959 the procedure was repeated in order to estimate the white grub population during late summer and autumn of the same year. The sampling units were dug in the same plots and fields as in the previous surveys. The plots were numbered for identification purposes and their size was marked on a sketch-plan for each area and also on 1 : 25 000-scale survey maps for the whole region of operations. The figures in Tables III and IV have been compiled from a total of 6900 sampling units.

These counts served for the calculation of

(a) The population density of cockchafers just before the flight period, their sex ratio and their distribution over the area.
(b) The population density of white grubs after the flight period and their distribution over the area.
(c) The rate of reproduction after the flight period, obtained by dividing the number of white grubs counted in a given plot or area by the number of cockchafers counted in the same plot or area before the flight period.
(d) The mortality in the three years between successive white grub or cockchafer counts.

214 Labelling procedures

The cockchafers collected in the area A or B by shaking their host trees or by operating light traps were confined in wire cages and transported to the field laboratory. There they were sexed and the males were marked with a leather dye. This consisted of an adhesive pure-white base with which other colours could be mixed. The beetles were labelled individually by applying the dye with small brushes as spots on the thorax or on the last abdominal tergum. When sufficiently dry, the beetles were packed into cardboard boxes in lots of about 200. These boxes were filled with wet vermiculate and then refrigerated to +4 - 6°C.

In 1962 the females captured in areas A and B were labelled with P^{32}. The following large-scale procedure was adopted. A solution of 8.9 ml NaH_2PO_4 having an activity of about 40 mc at 4 p.m. on 2 May was diluted with 5 l water and mixed with 10 l vermiculite. This mixture, containing 2.65 μc/ml, was poured in a plastic container with a cover. The female cockchafers were added to this labelling mixture in lots of several hundreds at a time. The beetles stayed in the mixture for a day, during which time the container was thoroughly shaken and rolled at intervals in order to secure complete contact between the beetles and the radioactive mass. When measured with a portable α - β - γ survey-meter (Tracerlab with a TGC-6 Probe) the freshly labelled beetles showed activities ranging from 200 to over 2000 counts/min. A count of 100 counts/min in an indi-

vidual was regarded as sufficient to distinguish between labelled and unlabelled beetles during screening procedures under unshielded conditions in the field, with a background of 30 - 50 counts/min. These P^{32}-labelled females were released only in area B.

215 Irradiation procedures

The beetles were stored and transported as indicated in sub-section 214 above to the nearest X-ray unit, which was at the Bürgerspital, Basle, 80 km from the field laboratory. The beetles were exposed in lots of 400 - 600 at a time. They remained in the same boxes of 13×18 cm surface by 5 cm depth for the whole trip, including exposure, until they were released in the fields. The X-ray unit used was a Siemens "Stabilivolt" operated at 200 kV and 20 mA. The focus distance and irradiation time were set for a dose-rate of 665 r/min or a dosage of 3325 (Fig.4) (Table V). Simultaneous dosage measurements were supervised by the radiophysicist of the Institute of X-ray therapy at the University of Basle. Each trip to and from the X-ray unit, including irradiation, took about four or five hours.

TABLE V

ESTIMATE OF THE COCKCHAFER POPULATION BASED ON SAMPLING IN GRASSLAND BEFORE THE FLIGHT PERIOD 1959 IN FIVE AREAS OF THE AJOIE FIELD EXPERIMENT

Area	Number of		Average number of cockchafers per m^2	Maximum quantity of males expected(1)
	plots	sampling units		
A	16	256	0.48	62
B	10	160	1.15	> 1000
C_1	6	96	0.12	16
C_2	7	112	0.54	65
C_3	7	112	1.00	130

216 Release of the irradiated cockchafers

The irradiated and marked males were usually released during the evening of the same day in area A. As the maximum flight activity of the natural population started twenty minutes after sunset, the irradiated beetles were released from the refrigerated cardboard boxes and poured into large wooden cases filled with a handdeep layer of coarse, moistened sawdust so as to be ready at sunset. In some exceptional cases we had to postpone the release to the next morning as the result of delays in handling or bad weather during the evening.

The releasing cases were exposed in grassland at about 250 m from the nearest border of wood described in sub-section 212. These cases were camouflaged with oak or beech twigs. As an example of such a release,

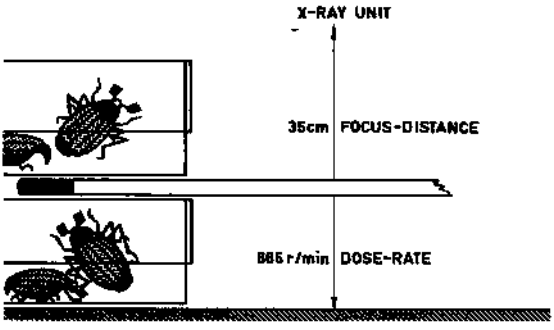

Fig. 4

Irradiation of male cockchafer for sterilization

Position of dosimeter during dosage measurements between two cardboard boxes each containing 200 males embedded in moistened vermiculite and kept below +6°C.

the one carried out on 12 May 1959 may be described. At 11.30 a.m. a total of 1504 males were exposed for release. Of these, about 850 left the cases during the first half hour. About 180 of them could be counted arriving at a prominent corner of the wood. Sunshine and a light northerly wind prevailed at this time; the temperature reading 2 m above the grass was 22°C.

At regular intervals the cases were checked for dead and remaining live males. The mortality was recorded. According to the counts taken at three different dates during the 1959 operations 89 - 94% of the beetles had left the releasing place on their own wings. The corresponding figures for the releases in 1962 were 74 - 98%. Bats and birds were consuming considerable numbers of cockchafers.

22 Results

221 Recapture of labelled males

Of 3109 males released in 1959 in area A, 202 individuals or 6.5% were recovered in light traps or collections along the woods. The corresponding figures for 1962 for the same area were: 8594 males released, of which 90 or 1% were recovered. Of the 2571 cockchafers labelled with dye and released in 1959 in area B, 22 or 0.9% were recaptured. Of the 6231 females labelled with P^{32} in 1962 in area B, 288 or 4.6% were recaptured. In 1959 the beetles in the two adjacent areas A and B had been labelled with different colours. In 1962 the males released in A were labelled with dye, whereas the females released in B were labelled with P^{32}.

No migration was observed in the two years either between these two areas A and B, or between those and any of the areas C.

222 Ratio of sterilized to normal cockchafers

Males were recaptured in the area in order to calculate the ratio of sterilized to normal cockchafers. During the period 10 - 14 May 1959 3109 irradiated males were released. In a sample of 514 beetles collected,

192 or 37.4% were labelled. For the last period recorded from 12 to 14 May 1959 the corresponding ratio was 52%. In 1962, for the first period recorded from 8 to 12 May, this ratio was 15 labelled to 18 recaptured or 83.3%. For the following period from 13 to 17 May 1962, the ratio was 30 to 30 or 100%.

223 Estimate of the cockchafer populations by the isotopic dilution technique

Cockchafers were recaptured in areas A and B in order to verify the estimates of the population as obtained by the sampling before flight had started. The formula used to calculate the population feeding and mating on the borders of the woods by the isotopic dilution technique was:

$$Q = q\,[(nl/r) - 1]$$

where Q = total number of individuals of the population;
q = total number of released and labelled individuals;
n = percentage of normally behaving individuals among total number released;
l = percentage of labelled individuals among total number released; and
r = percentage of labelled individuals in samples taken after release and intermingling.

The number of beetles released had to be corrected for those which lost the label, died or remained in the cases. The correction factor l was determined by the persistence of the label on the cockchafer. The persistence of the label had been checked at regular intervals on the cockchafers remaining in or near the releasing cages. The percentage of cockchafers which lost their labels could be reduced from 36% at the start of our operations to 0.2% at the end of them, with an average of 6%. The vitality of the released cockchafers is discussed in sub-section 215.

The estimate of cockchafer population may be compared in Table VII for the areas A and B in 1959 and in Table VIII for the same areas in 1962.

224 Effect of the first release of sterile males in 1959 on the subsequent white grub population

A remarkable reduction in the white grub population was obvious after the first release in A (see Table IX). The average number of larvae was $0.29/m^2$. All plots had infestations far below $20/m^2$, which may be regarded as a reliable indication of no damage in grassland. The white grub population in A dropped to 1/5 of that in the control areas and to 1/16 of that in area B.

The rate of reproduction calculated was less than unity in A, whereas in B and C the population had increased by factors of 2.6 and 2.7 respectively.

225 Effect of the second release of sterile males in 1962 on the subsequent white grub population

In 1962 not a single white grub was found in A. The number of cockchafers had increased in the other areas (see Table VI) as a result of an unusually low mortality rate in the period 1959 - 1962 (see Table IV), but

TABLE VI

ESTIMATE OF THE COCKCHAFER POPULATION BASED ON SAMPLING IN GRASSLAND BEFORE THE FLIGHT PERIOD 1962 IN FIVE AREAS OF THE AJOIE FIELD EXPERIMENT

Area	Number of plots	Number of sampling units	Average number of cockchafers per m^2	Maximum quantity of males expected (1)
A	12	196	0.1	13
B	10	160	1.3	> 1000
C_1	3	48	0.4	52
C_2	8	128	1.2	156
C_3	10	160	1.4	182

TABLE VII

ESTIMATE OF THE COCKCHAFER POPULATION IN 1959 IN THE AREAS A AND B DURING FLIGHT BY THE ISOTOPIC DILUTION TECHNIQUE BEFORE THE FIRST RELEASE OF STERILE MALES

Area	Period of observation	Sex	Individuals released	Labelled individuals recaptured	Correction[a] factors n (%)	Correction[a] factors l (%)	Proportion of labelled individuals (%)	Estimated population (No.)	Estimated population (l)
A	6-14 May	♂	3109	130	92	94	17.1	12620	25
		♀	1619	72	-	-	11.1	10934	22
Totals			4728	202	-	-	14.2	23744	47
B	10-14 May	♂	0	0	-	-	0	73258[b]	147
		♀	2571	22	97	94	5.7	38557	77
Totals			2571	22	-	-	1.96	116722	233

(a) Correction factor : n = % normally behaving individuals among total released.

l = % labelled individuals among total released.

(b) Estimate based on sex ratio of recaptured individuals ♂: ♀ = 1.9.

flight and oviposition were severely restricted by the unusually cold spring of of 1962. Egg development and hatching were prevented by drought. The reproduction rate in the whole region dropped below unity. The differences in infestation as between A and the other areas therefore appear less remarkable than they might have done.

TABLE VIII

ESTIMATE OF THE COCKCHAFER POPULATION IN 1962 IN THE AREAS A AND B DURING FLIGHT BY THE ISOTOPIC DILUTION TECHNIQUE BEFORE THE SECOND RELEASE OF STERILE MALES

Area	Period of observation	Sex	Individuals released	Labelled individuals recaptured	Correction[a] factors n (%)	Correction[a] factors l (%)	Proportion of labelled individuals (%)	Estimated population (No.) (l)	
A	4-16 May	♂	8594	90	97.8	93.5	76.3	1 699	3.5
		♀	0	0	-	-	0	2 005	4
Totals			8594	90	-	-	63.8	3 704	7.6
B	9-28 May	♂	0	0	-	-	0	24 271	48
		♀	6231	288	98.3	91.2	16.4	27 814	56
Totals			6231	288	-	-	9.58	52 085	104

(a) Correction factor : n = % normally behaving individuals among total released.

l = % labelled individuals among total released.

(b) Estimate based on sex ratio of recaptured individuals ♂ : ♀ = 1.9.

3 DISCUSSION

31 Prospects for successful application of the sterile-male technique to the elimination of white grub

Although it is possible to rear white grub and cockchafers in the laboratory, with the aid of a recent technique, in a shorter time than in nature (HORBER, 1959 [10]) it will hardly be possible to produce them in quantities large enough to outnumber the males of a natural population.

The basic strategy would be to capture males in areas where they are readily obtainable in large numbers and, after sterilizing them, to release them in the areas to be treated.

Thus the release of radiation-sterilized males enters the picture where a population is at a moderate-to-low level, either as a consequence of a regression in the course of natural gradation or following a more or less successful application of the other control measures. Even if not feasible for use alone, the method might be of importance in combination with others. Sterilized males might, for instance, be released to apply the coup de grâce to a population that has already been depleted by other control measures and in which the survivors could not be further reduced by any other practical means ; or this technique might be usefully applied to a population in the prodromal stages of an outbreak.

It is believed that this technique would not upset the natural balance or do direct harm to beneficial insects, bees, birds or fish, as has frequently

TABLE IX

EFFECT OF THE FIRST RELEASE OF STERILE MALES IN 1959 ON THE SUBSEQUENT WHITE GRUB POPULATION.

Estimate of the white grub population and of the rate of reproduction

Area	Number of plots	Number of sampling units	Average number of white grubs per m^2		Rate of reproduction	
A	14	224	2.29		0.52	
B	15	240	5.51		2.61	
C_1	7	112	1.29		12.00	
C_2	8	128	2.87	1.55	6.29	2.73
C_3	13	208	0.87		0.74	

TABLE X

EFFECT OF THE SECOND RELEASE OF STERILE MALES IN 1962 ON THE SUBSEQUENT WHITE GRUB POPULATION

Estimate of the white grub population and of the rate of reproduction

Area	Number of plots	Number of sampling units	Average number of white grubs per m^2		Rate of reproduction	
A	15	240	0		0	
B	11	176	0.91		0.77	
C_1	8	128	0.41		2.60	
C_2	10	160	0.78	0.57	0.82	0.65
C_3	10	160	0.50		0.36	

been observed after chemical treatments. No insecticide residues in grass and milk, nor off-flavour in potatoes or in other crops, need be expected, in contrast to experience with chemical treatments. This technique might also be useful in areas where cockchafers or white grubs have developed a high degree of resistance to insecticides.

32 Future problems

In the field experiment reported, the irradiation unit was situated far away from the release point of sterilized males. The irradiation time was therefore limited by the transport facilities and by the short time the unit was available for work with cockchafers, usually only at the end of a tight

schedule of the daily routine of X-ray therapy. The desirability of obtaining a mobile irradiation unit, with no other obligations while on duty for sterilizing work, is emphasized. The unit should be available in the immediate neighbourhood of the points of release. It would allow larger masses of insects to be treated in a much shorter time. This would enable larger areas to be covered or alternatively to be treated at higher infestation levels.

Furthermore, the basic strategy has to be considered and differentiated in a higher degree according to the ecological conditions or the farming system encountered in the area involved.

In our future work with cockchafer we plan to differentiate between areas with highly susceptible crops from other which tolerate a moderate degree of infestation. Areas where plants of zero tolerance are grown, e. g. potatoes, sugar beet, strawberries, vegetables, vineyards, orchards and nurseries, must be very carefully protected from white grub damage. Such areas have to be protected by an immediate reduction in the white grub population in the course of a single season. This involves manipulating the irradiation dosages so as to ensure a safe permanency of sterility in released males. In areas with grassland, cereals or other crops, which in contrast tolerate a higher infestation level, or where the cultures suffer from white grub damage only in the following season, the permanency of male sterility might be neglected in favour of the possibility of spreading a fair amount of sub-lethal genes among the surviving white grub population.

With regard to cockchafer and white grub, a field of application of the sterile-male technique appears to be in Alpine valleys, where the requirement of geographical isolation is usually fulfilled to some degree, where the terrain is difficult for the conventional control methods, and where the cultivated area is mostly limited to the bottom of the valley and is too small to support the high costs involved in treating the extended forests on both slopes. Sterile males released at well-chosen points would presumably operate better, by finding females and destroying their fertility in barren areas, than any surface-bound or airborne carrier of insecticide.

However, many more details of the sterile-male technique as applied to cockchafer, or any other insect pest, remain to be investigated more carefully. Thus it seems reasonable for leading workers to give such projects high priority in their schedule of activities, since this appears to be not merely another ingenious device of biological and technical progress in pest control, but in many respects a potential breakthrough.

33 Epidemiological studies facilitated by the sterile-male technique

It will be interesting to study the manner and speed with which areas such as area A, where white grub has disappeared after repeated releases of sterile males, become reinfested. Does reinfestation depend on immigration or on the multiplication of a few survivors? Another interesting task would be to determine the minimum size for viability in an area of a cockchafer population.

4 SUMMARY

Laboratory tests indicated an X-ray dose of 3000 r to be sufficient to induce sterility in male cockchafers. During two flight periods, sterilized males were released among a natural population in order to eliminate white grub in a mixed farming region of north-western Switzerland. In 1950 an outbreak of this pest was reduced by a chemical treatment. Gradation has been watched during every flight from 1953 to 1962.

In 1959 five areas, each with a surface of about 30 ha, were selected to serve as:

A treated area A, where males were captured, irradiated and released;
The "bank", B, where cockchafers were collected and the males were irradiated and released in A;
Three control areas, where undisturbed gradation was observed.

The males were irradiated in a therapeutical X-ray unit. Irradiated males were handpainted so that the ratio of sterilized males could be estimated by the isotopic dolution technique.

In 1959, for the first treatment, about 6 l of sterilized males were released in A, representing about 50% of the male population. The white grub infestation sampled in grassland dropped thereafter to about 1/5 of that in the other areas. Reproduction rate was less than unity only in A. A further reduction of the population in A to 1/10 of that in B and C was observed when the number of surviving cockchafers was estimated in 1962. The greatest mortality from 1959 to 1962 occurred in A.

In 1962, for the second treatment, a total of 17 l of irradiated males was released in A. At least 76% of the male population of A was sterilized. Subsequent sampling of the white grub population showed complete eradication in A. Some reduction was also observed in B and C as a result of drought in the whole region.

It has been demonstrated that the sterile-male technique may successfully be applied to an insect pest in an area which is not strictly isolated geographically, the females of which mate several times and the artificial breeding of which in large quantities is not feasible because of the long breeding cycle.

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REFERENCES

[1] BAUMHOVER, A. H., GRAHAM, A. J., HOPKINS, D. E., DUDLEY, F. H., NEW, W. D. and BUSHLAND, R. C., J. Econ. Entol. 48 (1955) 462-6.
[2] BUSHLAND, R. C. and HOPKINS, D. E., J. Econ. Entomol. 44 (1951) 725-31.
[3] BUSHLAND, R. C. and HOPKINS, D. E., J. Econ. Entomol. 46 (1953) 648-56.
[4] CATCHESIDE, D. G., Advances in Genetics 2 (1948) 271.
[5] GROSCH, D. S., Ann. Rev. Entomol. 7 (1962) 81-106.
[6] HILCHEY, J. D., Radiation Preservation of Food, US Army Quartermaster Corps, Washington, D. C. (1957) 240-266.
[7] HORBER, E., Mitt. Schweiz. Landw. 2 (1954) 17-52.
[8] HORBER, E., Landw. Jb. Schweiz NF 4 (1955) 1-14.
[9] HORBER, E., Mitt. Schweiz. Landw. 6 (1958) 33-43.
[10] HORBER, E., Landw. Jb. Schweiz NF 8 (1959) 361-370.
[11] HORBER, E., Landw. Jb. Schweiz NF 10 (1961) 635-669.
[12] KNIPLING, E. F., J. Econ. Entomol. 48 (1955) 459-462.
[13] KNIPLING, E. F., Sci. Amer. 203 (1960) 54-61.
[13] KNIPLING, E. F., Sci. Amer. 203 (1960) 54-61.
[14] LEA, D. E., Actions of radiations in living cells, Cambridge University Press, Cambridge (1947) 402 pp.
[15] LINDQUIST, A. W., J. Econ. Entomol. 48 (1955) 467-469.
[16] MULLER, H. J., J. Econ. Entomol. 40 (1940) 1-66.
[17] MULLER, H. J., J. Genet. Cold Spring Harbor Symposia Quant. Biol. 9 (1941) 290-308.
[18] SCHENKER, P., ZMB-Berichte No. 47 (Liebefeld-Bern) (1954) 8 pp.

DISCUSSION

M. FERON: I would like to mention that the cockchafer is a particularly favourable insect for this type of experiment. Professor Stone indicated earlier that a strict isolation of the areas concerned is needed in order to obtain success*. In the case of the cockchafer, the female comes out of the fields to go to the trees for mating, and afterwards it goes back exactly the same way in order to oviposit. The movements of the females are thus restricted and this insures a certain behavioural isolation. The males, of course, do not behave in the same way, since they do not lay eggs. I think this contributes considerably to the success of such an experiment.

E. HORBER: That is quite correct. The behavioural studies of my colleagues Couturier, Roberts and Schneider show that at moderate population levels behavioural isolation can be obtained even without geographical isolation.

W. KLOFT: In regard to the sex ratio of cockchafers, the figures in Table I show more males than females. Several times in Würzburg we collected Melolontha vulgaris and we always found an exact 1:1 sex ratio. But in your case the insects were caught with light traps. Perhaps that explains the different results obtained.

E. HORBER: Yes, these figures given in my report apply only to insects caught in light traps, during the flight period; moreover, they are possibly peculiar to the locality where the light trap was operated. The sex ratio of a natural, undisturbed population can best be checked by digging

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* See discussion of STONE, W. E., Effects of ionizing radiation on insects and other arthropods, these Proceedings.

the beetles out just after metamorphosis during the autumn, before the flight period. The ratio may then be expected to be exactly 1:1. In our survey work during many years at different localities it was always exactly or very nearly 1:1 as verified by the χ^2 test. During the flight period, however, a continuous dynamic process is going on whereby the sex ratio may change very rapidly depending on several factors, e.g. phase of oviposition, higher mortality rate of the males, etc.

G.B. VIADO (Chairman): In the Philippines we have several species of white grub which are very destructive to crops. Extensive surveys were carried out many years ago because of the damage they do to sugar cane. We collected sacks full of these adult beetles by shaking them from trees and shrubs and we found a sex ratio of about 2 : 1 in favour of males.

A TECHNIQUE OF CULTURING THE OLIVE FLY, DACUS OLEAE GMEL., ON SYNTHETIC MEDIA UNDER XENIC CONDITIONS

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Abstract — Résumé — Аннотация — Resumen

A TECHNIQUE OF CULTURING THE OLIVE FLY, DACUS OLEAE GMEL., ON SYNTHETIC MEDIA UNDER XENIC CONDITIONS. Five generations of Dacus oleae have been cultured on an agar-dehydrated carrot base medium that contains an enzymatic protein hydrolysate of soya or casein, brewer's yeast, choline chloride and olive oil. Although the culture technique is xenic, an attempt is made to control microorganisms by chemical means. A bacterial species replaced the normal symbiote within D. oleae and was believed to be essential to maintaining the stock, but two generations of D. oleae have been cultured without any bacteria being present in the sites normally occupied within the larva or adult by its typical symbiote. Streptomycin is now being incorporated into the adult food to control bacterial infection of the eggs. Normal larval development, size and reproduction of D. oleae is obtained.

Mass culture is possible using the larval medium developed, but further research is necessary to find a faster method of placing the eggs on the medium. Further screening of mould inhibitors is desirable, as well as seeking cheaper substitutes for the medium.

MÉTHODE D'ÉLEVAGE DE LA MOUCHE DE L'OLIVE (DACUS OLEAE GMEL.) EN XÉNIE SUR MILIEUX SYNTHÉTIQUES. On a élevé cinq générations de Dacus oleae sur un milieu à base de gélose et de carotte déshydratée contenant un hydrolysat enzimatique de protéines de soja ou de caséine, de levure de bière, de chlorure de choline et d'huile d'olive. Bien que l'élevage se fasse en xénie, on s'efforce d'enrayer le développement des microorganismes à l'aide de moyens chimiques. Une espèce bactérienne a remplacé le symbiote normal chez D. oleae et sa présence semblait indispensable dans le lot d'élevage, mais on a élevé deux générations de D. oleae sans qu'aucune bactérie soit présente aux emplacements normalement occupés chez la larve ou l'adulte par le symbiote caractéristique. Pour éviter la contamination des œufs par les bactéries, on incorpore maintenant de la streptomycine au régime alimentaire des adultes. On a obtenu un développement larvaire normal et des insectes de taille normale, qui se reproduisent normalement.

Le milieu larvaire qui a été mis au point permet l'élevage en masse, mais il faudrait procéder à de nouvelles recherches pour découvrir une méthode plus rapide pour placer les œufs sur ce milieu. Il faudrait perfectionner les inhibiteurs de moisissure et mettre au point d'autres milieux d'un prix de revient moins élevé.

МЕТОДИКА КСЕНИЧЕСКОЙ КУЛЬТИВАЦИИ МАСЛИННОЙ МУХИ DACUS OLEAE НА СИНТЕТИЧЕСКОЙ СРЕДЕ. Пять поколений D.oleae культивировали на среде агар-обезвоженная морковь, содержащей энзиматический гидролизат соевого или казеинового белка, пивные дрожи, хлорид холина и оливковое масло. Несмотря на ксеническую методику культивации, сделана попытка контролировать микроорганизмы химическими средствами. Бактериальные разновидности заменяли внутри D.oleae нормальный симбиот, что, как полагали, являлось важным для сохранения вида. Однако у двух поколений D.oleae не обнаружено каких-либо бактерий в участках, обычно занимаемых внутри личинки или взрослой особи типичным симбиотом. Стрептомицин в настоящее время включается в состав пищи взрослой особи для предупреждения бактериальной инфекции яиц. Личиночное развитие, размеры и размножение D.oleae были нормальными.

С помощью разработанной личиночной среды возможна массовая культивация, однако необходимы дальнейшие исследования для создания более быстрого метода размещения яиц на среде. Желателен дальнейший отбор ингибиторов плесени, а также отыскание более дешевых заменителей среды.

UNA TÉCNICA DE CRÍA DE LA MOSCA DEL OLIVO (DACUS OLEAE GMEL.) EN UN MEDIO SINTÉTICO EN CONDICIONES XÉNICAS. Se han criado cinco generaciones de Dacus oleae en un medio a base de agar

y de zanahoria deshidratada que contenía un hidrolizado enzimático de proteínas de soja o de caseína, levadura de cerveza," cloruro de colina y aceite de oliva. Aunque la técnica de cría es xénica, se ha tratado de controlar químicamente el desarrollo de los microorganismos. Una especie bacteriana ha reemplazado el simbiota normal del D. oleae y su presencia parecía indispensable, pero se han criado dos generaciones de D. oleae sin bacteria alguna en los lugares que el simbiota característico ocupa normalmente en la larva o en el adulto. Para evitar la infección bacteriana de los huevos se añade ahora estreptomicina al alimento de los adultos. El desarrollo de las larvas y el tamaño y la reproducción de los insectos son normales.

El medio preparado para las larvas permite la cría en masa, pero sería necesario efectuar nuevas investigaciones a fin de descubrir un método más rápido para colocar los huevos en ese medio. También convendría estudiar nuevos anticriptogámicos y encontrar productos que permitieran preparar un medio menos costoso.

INTRODUCTION

Trypetid pest species are likely candidates for control or eradication by releasing sterile (lethal) males [1-9]. This technique is possible if a species can be mass-cultured.

Five trypetid species can to date be mass-cultured on prepared media not containing their natural host tissue. These species are the Mexican fruit fly, Anastrepha ludens Loew [10], the Mediterranean fruit fly, Ceratitis capitata Wiedeman [11-15], the oriental fruit fly, Dacus dorsalis Hendel [11-13], the melon fly, Dacus cucurbitae Coq. [13], and the Queensland fruit fly, Dacus tryoni Frogg [16, 17].

Dacus oleae Gmelin is also an important pest species. It causes extensive economic losses to olive growers in the Mediterranean region. In Greece alone, it is estimated to cause losses of about US $15 million annually [18, 19].

The Greek authorities became interested in conducting an experiment using the irradiation-of-male method of control against the olive fly on an island. Greece is ideally suited for such an experiment because of its numerous isolated islands bearing olive trees. The Greek Atomic Energy Commission consulted the United States Atomic Energy Commission as to the feasibility of such a test and the latter Commission sent two entomologists to Athens to investigate the problem.

These visiting entomologists met with a special committee consisting of entomologists and biologists from the Greek A.E.C., the University of Athens, the Department of Plant Pathology of the Ministry of Agriculture, the College of Agriculture of Athens and the Benaki Plant Pathological Institute. The committee was not only concerned with the sterile-male technique of control but outlined a research programme, (which included ecological studies, a search for new adult attractants and intensifying work on other methods of control), since any type of control that would reduce the average density of the olive fly would make it less difficult to obtain favourable overflooding ratios of released lethal males.

It was clear from the discussions with the committee that the olive fly would be amenable to the sterile-male technique. The monophagous nature of D. oleae, which attacks only olives, and its low abundance during certain periods of the year are attributes that perhaps make it better suited for the sterile-male release method than the other more tropical species of trypetids that have wider host ranges and more generations. The olive fly is indeed polygamous and more so than the other trypetids under investigation at

present. However, it is thought now that polygamy is not a critical factor for the lethal-male release method [20, 21].

Preliminary studies with dosages of irradiation necessary to induce dominant lethality in the sperm have been determined for the puparia, and cage tests with competitive normal males have given favourable results [4]. Radiation studies with other stages and puparia of D. oleae have recently been made in Greece [22].

It was plain to the committee in Greece that the main problem to be solved before an island test could be made using sterile D. oleae males was first to develop a mass-culture technique. Simultaneously further research on irradiation dosages and a search for effective adult attractants should be made.

The "Olive Branch Enterprise", as the committee had named their programme, requested an expert on fruit-fly nutrition through the International Atomic Energy Agency. In November 1961, K. S. Hagen was obtained from the University of California at Berkeley, California, and work was begun on the culture of the fly at the College of Agriculture in Athens.

RESEARCH APPROACH

There are at least three approaches that can be made toward culturing D. oleae. They can be classified as axenic, monoxenic and xenic. These terms refer to the number of organisms associated with the species that is under investigation as to its nutritional requirements [23].

The axenic approach to culturing D. oleae larvae requires that no other species of organism be present either in the larva or in its substrate. MOORE [24, 25], using aseptic techniques, attempted to use this approach, and was able to obtain adults. The developmental period was slower for the larvae than normal, and the resulting adults were reproductively weak.

It is possible with the technique Moore used that a monoxenic condition existed, i.e. that besides D. oleae one other species was involved in the culture, a bacterial symbiote. An extra-cellular symbiote, Pseudomonas savastanoi Smith, is found in nearly all wild D. oleae and is transmitted from generation to generation [27-29]. Even though Moore surface-sterilized the D. oleae eggs, some bacteria could have already entered the egg at the time of deposition.

The olive-fly larva with its symbiote within an olive is approaching a monoxenic condition, for usually the olive appears to be quite sterile at the larva feeding site. The larva prefers to eat undisturbed tissue as it tunnels through the fruit.

Recent experiments, which will be reported elsewhere in detail by the senior author, conclusively showed that, when adults of D. oleae which have been reared from olives are fed streptomycin along with the regular laboratory adult diet, eggs deposited in olives hatched. However, nearly all the larvae died in the first-instar stage after tunnelling considerable distances in the olive. No larvae attained the third instar.

Eggs obtained from these same adults and placed on an artificial medium containing an enzymatic protein hydrolysate completed their development normally, and the resulting adults deposited viable eggs. Thus the role of Pseudomonas savastanoi as a symbiote is perhaps to hydrolyse the protein

in the olive tissue ingested by the larva, making available some essential amino-acid or acids, or possibly to synthesize an amino-acid or acids lacking in the olive.

The diet that MOORE [25] employed for the larva was similar to the aseptic diets used for some other fruit-fly species [26], except that it contained a higher level of protein and sitosterol was used in place of cholesterol. The fact that no hydrolysed protein was in the diet Moore used possibly indicates that the symbiote was present in the larvae.

The approach used in Greece was a xenic one, which infers that the number of associated organisms in the larva or in the substrate is unknown. Since it would be difficult to mass-culture a trypetid species under aseptic conditions, a xenic approach is the only practical one. However, it is imperative that harmful contaminants be controlled. The technique of using an acid pH and mould inhibitors to control micro-organisms developed for the culture of other trypetids [26] was therefore used for D. oleae.

The rather acid pH of the medium controls many bacteria, including the normal symbiote P. savastanoi, but permits at least one other bacterial species to replace it. These alien bacteria may become pathogenic in older females and reduce fecundity by reducing the lifetime. Streptomycin was therefore incorporated in the adult food in an attempt to prevent bacteria from becoming established in the special morphological sites evolved to house the typical symbiote. The cycle of P. savastanoi and its sites in D. oleae have been well known since the classic work of PETRI [27]. (For other pertinent papers see [28, 29]).

Though the culture approach described below is a xenic one, it is nearing an axenic condition at least insofar as D. oleae harbours bacteria in its unique symbiote morphological sites. The inclusion of hydrolysed protein in the larval medium apparently permits D. oleae to develop without its symbiote.

MATERIALS AND METHODS

The temperature was usually 25 ± 1°C and the relative humidity was 75 ± 20%. Later the relative humidity was held at about 70% with the aid of a humidifier and a humidistat.

Adult handling

The type of cage used for stock flies is one that has been used for years in United States fruit-fly laboratories. About 200 flies were held in the 30-cm-cube cages (Fig. 1). The cages were placed near east-facing windows and exposed to normal diurnal light.

The adult food containing an enzymatic protein hydrolysate of yeast with a carbohydrate, and at times with brewers' yeast, is quite similar to the diets used in the culture of other trypetid species [30-32], and was effective for D. oleae egg production [25, 33]. The adult diet now used as a matter of routine in Greece is shown in Table I.

The diet shown in Table I, without streptomycin, was used to obtain eggs for most of the tests made. Only in later tests was streptomycin used and this will be indicated where appropriate.

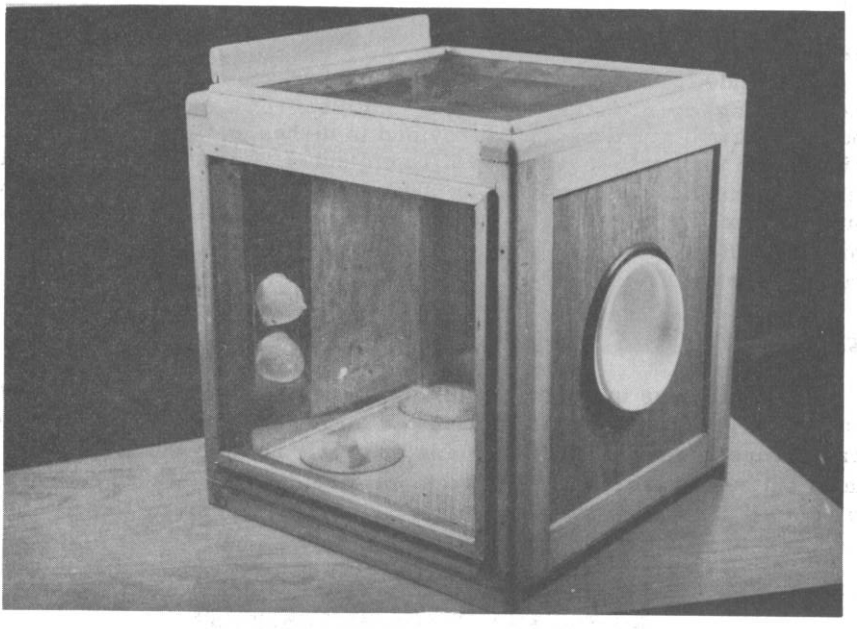

Fig. 1

Cage for adult Dacus oleae showing ovipositional sites (the paraffin-wax domes) and the feeding site (the wax paper) sealed inside on the front glass

TABLE I

COMPOSITION OF ADULT DIET FOR DACUS OLEAE

Substance	Amount (g)	Amount (ml)
Enzymatic protein hydrolysate of yeast*	20.0	
Sucrose	80.0	
Choline chloride*	0.1	
Streptomycin sulphate *	0.5	
Water		100.0

* Nutritional Biochemicals Corp., Cleveland, Ohio.

It is imperative that the adult food be supplied to the flies in a fluid form. Placing solid enzymatic protein hydrolysate yeast in the cage along with solid sucrose and water separately, the method used for feeding other fruit-fly species, is not effective with D. oleae. This species feeds very little on solid protein hydrolysates. It will feed, to some extent, on solid sucrose.

It appears that protarsal contact with the food is necessary to obtain a feeding response.

The liquid food is brushed in streaks onto wax paper which is sealed to the glass cage front facing the light. Solid sucrose and a water-soaked cotton-wool wad are separately provided in dishes on the floor of the cage (Fig. 1). In the smaller test cages (15 cm cube), where five pairs of flies were often used for determining fecundity, the food was provided in triplet form on wax paper on the floor of the cage, and once a day the food was sprayed with a fine mist of water. In the larger cages, it is necessary to brush on the food twice a day.

Egg handling

To obtain eggs in a suitable form for manipulation or counting, two domes of thin paraffin wax were used per large cage (Figs. 2 and 3). These domes were sealed to a slip of glass by gently heating the glass on a hot plate, but before sealing the glass a small wad of water-soaked cotton-wool was placed under each paraffin dome. This was necessary to prevent the

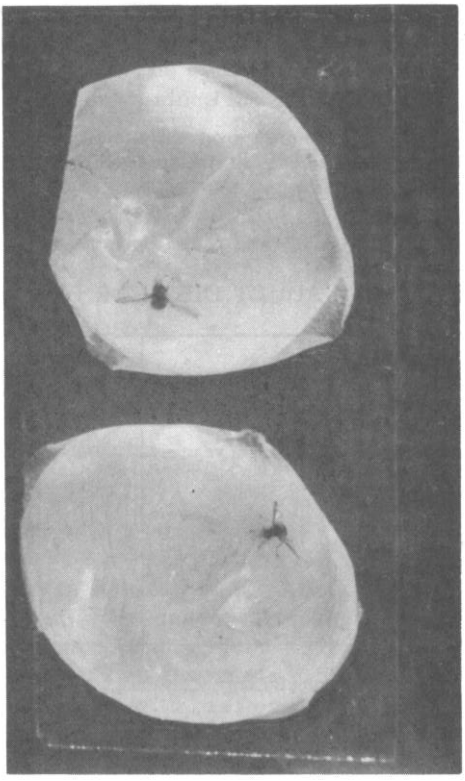

Fig. 2

Top view of paraffin-wax domes sealed to a glass slip showing Dacus oleae ovipositing

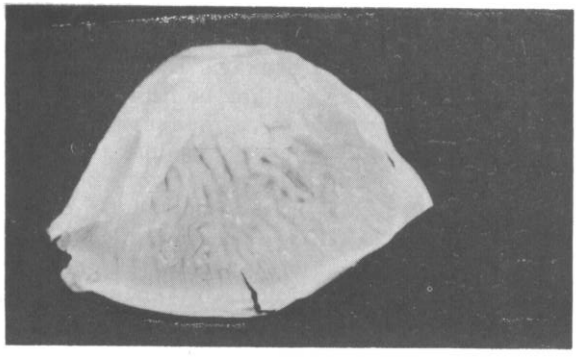

Fig. 3

Under-surface of paraffin-wax dome showing eggs of Dacus oleae at deposition sites

eggs from drying. The glass slip with the domes was fastened with cellulose adhesive tape to the front glass next to the food paper (Figs. 1 and 2). Fewer eggs were obtained if the domes were exposed on the floor of the larger cages.

The paraffin domes are easily made by using paraffin wax with a melting point of 52-53°C, or mixtures of paraffins with different melting points can be used as long as the resulting melting point is between 52 and 55°C. The best results have been obtained by adding 5-10% beeswax to the paraffin wax melting at 52-53°C. The paraffin is heated to between 70 and 90°C. A wooden mould of the size and shape of a hen's egg is first dipped into a thick soap solution and then plunged quickly first into the hot paraffin and then into water. The hardened paraffin shell is now easily pulled off the mould.

It is necessary to make new domes every day, for the used ones usually break when the eggs are removed from the under-surface (Fig. 3); also the domes become so perforated with ovipositional punctures that the eggs may become too dry.

Plastic moulds or thick wax forms, with or without ready-made punctures, have been used successfully with other trypetids being cultured but are not utilized by D. oleae for oviposition. This species does not oviposit in an existing hole, but drills, or attempts to drill, a new hole each time it deposits its single egg. Thus a very thin, easily penetrated material must be used in order to recover good eggs.

Often oviposition is attempted on any smooth, clean surface, like glass, and frequently an egg is deposited on this surface. These eggs often fall to the bottom of the cage and are lost because they soon become dry. Cages with screen bottoms permit the eggs to fall through into pans of water, and these eggs can be used. However, these eggs can easily become contaminated with microorganisms.

The paraffin domes are placed in the cages each morning and are removed in the afternoon or the next morning. The shorter the exposure to oviposition the better, since the range of hatching would be shorter and more uniform.

The eggs from the domes are collected in several different ways. The most rapid method is by dipping the domes into distilled water. The dislodged eggs fall to the bottom of the beaker. Excess water is decanted off, and the eggs, with some water, can either be filtered and caught by a fine nylon organdy, or , if the eggs are to be measured, they are drawn and pipetted into a funnel that has its tip pressed against a piece of nylon organdy which rests upon the surface of a large rubber cork held by a clamp. The eggs are measured volumetrically in the funnel tube and spread out over the surface of the organdy by brushing. The nylon pad with eggs is placed on moist filter paper and kept moist until hatching begins. For many experiments the eggs were removed individually by brush from the domes and placed on moist filter paper.

The incubation period is about 2.5 d at 25°C. The Petri dishes containing the eggs on wet filter paper must be clean and kept as free as possible from invasion by microorganisms.

A copper chloride solution of 1:1000 was used for a time in the egg dishes to control moulds and yeasts, but this was discontinued since the adults resulting from the eggs often died prematurely.

At present some mould inhibitors are being tested in the egg-holding dishes in an attempt to control yeasts and moulds that arise from egg placement on the medium.

Eggs exhibiting larvae with visible mouth hooks, and larvae which have just hatched, are placed on the media. Larvae one day old become too weak to be used. In earlier tests the egg pad was placed upon the smooth surface of the medium, and the larvae would enter the medium through the cloth mesh. However, eggs in contact with each other often would not hatch. The oil from the medium seeping up through the pad may have accounted for this egg mortality.

A higher percentage of hatch and puparial recovery is obtained when the egg or larva is placed individually directly upon the medium by brush. There is less chance of the medium becoming heavily contaminated than when an egg pad is placed on the medium. The water carried by the nylon egg pad evidently dilutes the mould inhibitors and alters the pH, allowing growth of unwanted microorganisms.

The eggs or larvae are placed in rows on the medium adjacent to shallow grooves made in the medium with the tips of a pair of forceps (Fig. 4). These grooves are necessary to permit the larvae to enter the medium easily.

Larval media

Over 700 different larval media were tested. Many did not permit any larval development. The medium which has given the best results to date is shown in Table II. This medium will be referred to hereafter as "the standard medium".

The medium is prepared by bringing all the ingredients mixed in water to a boil except the hydrochloric acid and the dehydrated carrot. After the mixture has been allowed to cool to about 70°C, the hydrochloric or citric acid is added. This mixture is then homogenized in a high-speed blender for about one minute. The powdered dehydrated carrot is added and stirred in slowly with a mixer. If the dehydrated carrot is homogenized with the

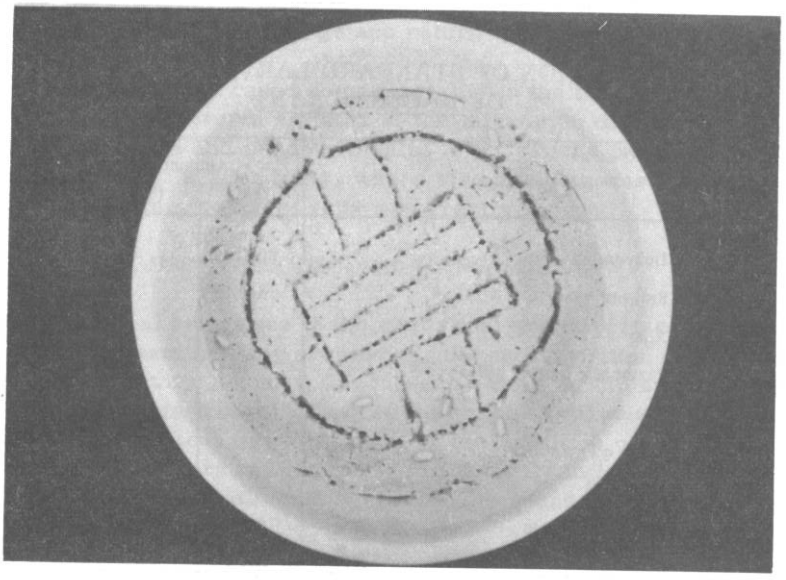

Fig. 4

Puparia of Dacus oleae on surface of larval medium
where they pupated after the larvae came to the surface
The grooves in the medium enable the larvae to enter the medium
after hatching from eggs placed on the flat upper surfaces.

other ingredients the medium becomes so dense that the larvae appear not to be able to utilize it.

If no blender is available, the above procedure should be followed but the mixture should be well stirred when the acid is added, and the carrot powder should be stirred in afterwards until a smooth consistency is obtained. The pH can be easily ascertained fairly closely by using indicator paper.

The media were tested in various containers. The container found most convenient for most experiments was a thin plastic dish (Fig. 4) 9 cm in diameter at the top and 4 cm deep. About 50 ml of medium was poured into the dish. The depth of the medium was usually around 1 cm. Three such dishes were used for each test medium. The medium must not be allowed to dry, therefore another larger-diameter plastic dish was placed tightly over the medium dish.

The larvae enter the medium and disappear. Very little activity is observed until a day or two before pupation. At this time large holes begin to appear in the surface of the medium, and outlines of galleries can be seen through the thin sides and bottom of the dish. The larvae come to the surface of the medium to pupate.

The first larvae complete their development usually 10-13 d at 25°C on the better media. On the first or second day following the first puparia product, the peak number pupate, and within five days from the appearance of the first puparia all larvae have usually completed their development. The speed of larval development is an indication of the nutritional quality of the medium.

TABLE II

COMPOSITION OF STANDARD LARVAL MEDIUM OF DACUS OLEAE

Substance	Amount (g)	Amount (ml)
Dehydrated carrot 1/	25.0	
Brewers' yeast	15.0	
Agar	2.0	
Enzymatic protein hydrolysate of soya 2/	6.0	
Choline chloride	0.05	
Olive oil (virgin)		15.0
Tween 80		5.0
Sodium benzoate	0.3	
Butoben 3/	0.05	
2 N hydrochloric acid 4/		7.0
Water		125.0
	48.4	152.0

1/ Puccinelli Packing Co., Turlock, California.
2/ Nutritional Biochemicals Corp., Cleveland, Ohio.
3/ n-butyl parahydroxybenzoate (Merck).
4/ Enough acid to adjust the medium to a pH reaction of about 4.1.

Puparia handling

The puparia are collected daily from the medium and placed in Petri dishes along with a small wad of water-soaked cotton-wool. This added moisture is important to normal pupation during the first few days after formation.

The weight of the puparia is also another index as to the nutritional quality of the medium. Since the puparial weight may vary with age, one-day-old puparia were weighed and recorded. It appears that the larger puparia in any one test are the first ones produced.

The number of puparia recovered versus the number of eggs that hatched on the medium or the number of larvae placed on the medium does not necessarily indicate nutritional quality, for it is believed that the physical nature of the media, and the method of egg handling at the time of placement, influence survival.

The greatest mortality occurs during the first larval instar. Suffocation due to oil blocking the tunnels may account for most mortality. Dacus oleae

larvae do not back out of tunnels, whereas some other trypetids living in rather fluid media can submerge and return their spiracles to the surface for air [34].

Alien bacteria may cause some mortality in the first-instar larvae, for great numbers of bacteria situated at the micropyle of the egg might gain entry into the larva and be detrimental to it. These bacteria are not the normal symbiote. In later tests, where streptomycin was fed to the adults, the bacterial contamination of eggs was greatly reduced.

RESULTS AND OBSERVATIONS REGARDING ADULTS

The biological responses to the adults that are important to culture and can be observed extrinsically are mating, the preoviposition period, fecundity, fertility and longevity. Some data concerning these functions are from adults reared from puparia from field-collected olives (Figs. 5 and 6). The other data are from flies that were produced on the artificial media.

Fecundity, fertility and longevity are not only influenced by adult diet, but may also be greatly affected by larval diets and uncontrolled bacterial activity in the adult.

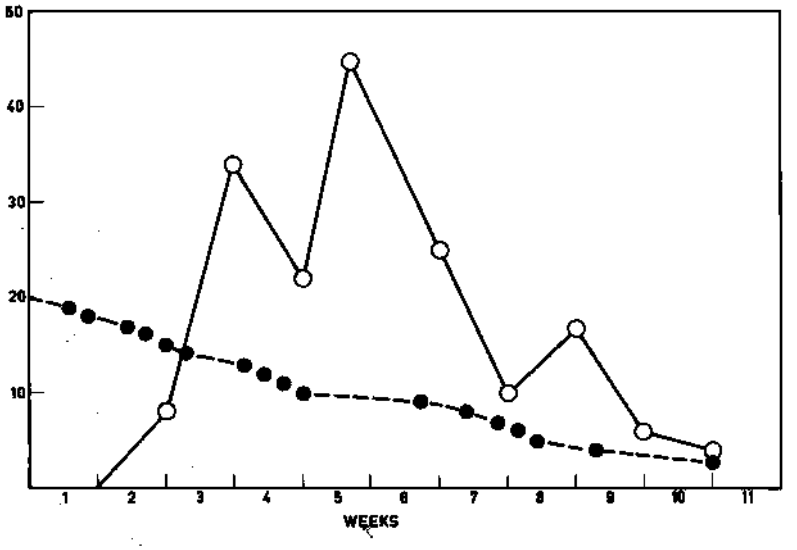

Fig. 5

Average fecundity per Dacus oleae female per week and longevity of 20 females reared on olives

○ No. of eggs per female per week
● No. of living females

Mating

Experiments showed that the males will mate effectively if fed only carbohydrate solutions. In this respect, D. oleae is unlike D. dorsalis [31], for in the latter species both sexes must ingest protein in their adult diet in

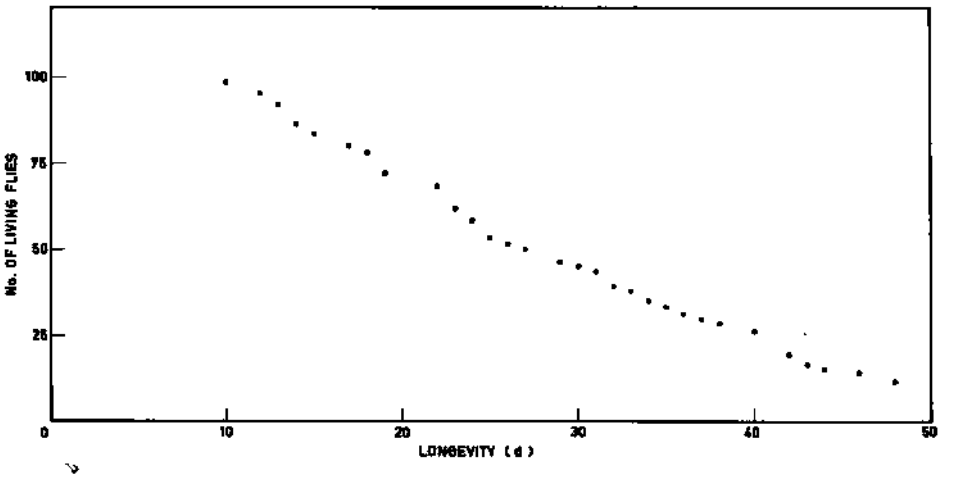

Fig. 6

Longevity of 100 Dacus oleae adults that emerged from field-collected puparia and fed on yeast hydrolysate + sucrose + choline chloride + water

order to copulate. The male of D. dorsalis shows viable spermatozoa in the testes early but will not mate at any age until it ingests protein and requires at least five days after being exposed to the protein before it will attempt to copulate.

The males of D. oleae usually begin mating in four days after emergence, but initial mating on the part of the male may occur earlier or later depending upon the attraction of the female. Long preoviposition periods are correlated with correspondingly late initial matings. Mating is apparently triggered by decreasing light intensities associated with the approach of the twilight period. This mating response to light-change seems to be the same as that reported for D. dorsalis [37] and D. tryoni. Thus it is important that D. oleae be kept in rooms where natural diurnal light is available.

A perceptible sound or call is made by the male in the approach to a susceptible female [35]; this occurs also in other Trypetidae [36]. There can be many matings by the same individuals of D. oleae during their life. The frequency of remating is greater in D. oleae than in the five other trypetids now being mass-cultured. Only during the second mating attempt, which occurred on the second evening following the first mating, was there any reluctance to accept an approaching male. However, during each successive evening for 10 days mating could be observed. It was not determined whether any spermatozoa were transferred after the first mating.

Preoviposition period

The length of this period can vary considerably. The season of the year, the nutrition of the larvae and the nutrition of the adult influence the length of the preoviposition period.

During November, December and January the preoviposition period was the longest in the laboratory, where the temperature was more or less

TABLE III

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF ENZYMATIC PROTEIN HYDROLYSATE OF SOYA[1] UPON THE DEVELOPMENTAL PERIOD OF THE LARVAE, THE WEIGHT AND RECOVERY OF PUPARIA AND THE PREOVIPOSITION PERIOD OF ADULTS

Concn soya hydrolysate[2] (g)	No. fertile eggs[3]	Minimum period to pupation (d)	Wt. of puparia (mg)		Puparia recovered (%)	Fly emergence (%)	Minimum preoviposition period (d)
			Mean	Range			
5	76	13	6.1	5.9-6.3	39	100	23
6	87	11	6.2	5.7-7.0	32	100	13
7	79	11	6.0	5.7-6.4	20	100	13
8	99	11	6.1	5.7-6.7	45	100	13

1/ A product of the Nutritional Biochemicals Corp., Cleveland, Ohio, USA.
2/ Other ingredients as shown in the standard medium (Table II).
3/ Number of eggs that hatched on the medium.

TABLE IV

THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF ENZYMATIC PROTEIN HYDROLYSATE OF CASEIN[1/] UPON THE DEVELOPMENTAL PERIOD OF THE LARVAE, THE WEIGHT OF THE PUPARIA AND THE PREOVIPOSITION PERIOD OF ADULTS

Concn[2/] casein hydrolysate (g)	Amount[2/] 2N HCl used (ml)	No. fertile[3/] eggs	Minimum period to pupation (d)	Wt. of Puparia (mg)		Puparia recovered (%)	Fly emergence (%)	Minimum preoviposition period (d)
				Mean	Range			
2	6	95	12	6.1	5.4-6.4	25	100	35
4	7	75	11	6.5	6.4-7.0	30	100	17
6	7	87	10	6.1	6.0-6.2	25	100	16
8	7	100	10	6.0	5.7-6.2	33	100	-
8	8	98	9	6.1	5.9-6.2	21	100	16

1/ Product of the Nutritional Biochemicals Corp., Cleveland, Ohio, USA.
2/ Other ingredients as shown in standard medium (Table II), but casein hydrolysate used in place of soya hydrolysate.
3/ Number of eggs that hatched on the medium.

constant. Either the photoperiod or the amount of light intensity per day may be involved. Perhaps the light intensity influences the feeding rate.

The data shown in Tables III and IV indicate that the concentration of protein hydrolysate in the larval medium may influence the adult preoviposition period. The higher protein hydrolysate concentrations are correlated with shorter preoviposition periods. Thus in D. oleae there is definitely metabolite transfer from larval feeding that influences egg production.

Adult nutrition has an important influence on the preoviposition period in other trypetids [31, 32], and in D. oleae some studies have been made by MOORE [33].

Fecundity

The number of eggs produced by D. oleae is apparently less than in the five species now being mass-cultured. The fact that only one egg is deposited per oviposition shows the olive fly to be strikingly different from the other species cultured, which deposit about 20-60 eggs per deposition. Even though the olive fly appears to live longer than the other species, its total fecundity does not seem to approach that of the other five species. A rough comparison would be of the order of 300:2000 under laboratory conditions. MOORE [33] obtained as many as 700 from some individuals of D. oleae.

The data shown in Fig. 5 are the number of eggs deposited per female per week by adults reared from olives. The average total number of eggs deposited per female was 183, the range being 99-276. In this case the flies were held in small 15-cm-cube cages.

An average of about one egg per female per day is obtained from the larger stock cages (30 cm cube) as compared with 2.5 eggs per female per day obtained over a 10-wk period. The proximity of the female to the feeding and ovipositional sites may account for the increased fecundity, and there is less interaction between flies in the small cages.

The presence of an alien species of bacteria in the morphological sites of the normal symbiote can greatly influence fecundity by shortening longevity. What appears to be the same bacterial species is in some individuals under control while in others the increase is so great in the bacterial pockets of the ovipositor normally occupied by P. savastanoi that blockage of the ovipositor occurs, which prevents both oviposition and excretion and results in the premature death of the female. A typical symptom of this condition is manifested by an extruded ovipositor which cannot be retracted. Pressing the tip of the extruded ovipositor on a microscope slide will reveal great masses of bacteria. There is no general septicaemia in the adult at the time of death. The influence of the pathogenic condition in the female on egg production is shown in Fig. 7.

Five generations of D. oleae were cultured in the presence of what was apparently the same species of bacteria. These bacteria accounted for erratic fecundity as between individuals. The variability in pathogenicity may be a reflection of the density of bacteria that made an original entry into the larvae from the egg, for the number of bacteria surrounding the micropyle of the egg would vary greatly. However, since no identification

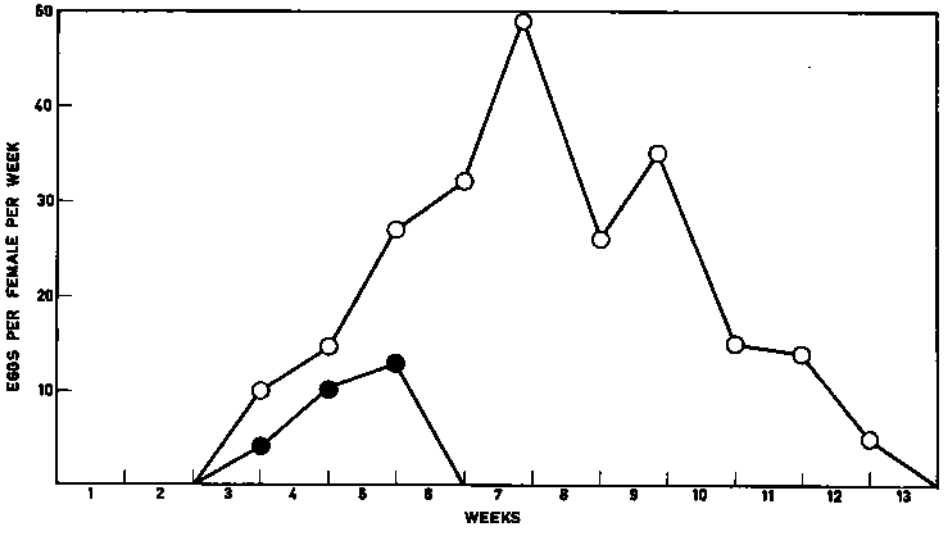

Fig. 7

Average fecundity per Dacus oleae female per week
for flies cultured on the standard medium,
except that 5 g enzymatic protein hydrolysate was used

○ Without pathogenic bacteria
● With pathogenic bacteria

The sample population consisted of 13 flies of which 5 were observed
to be infected with pathogenic bacteria and 8 were not infected.

of the bacteria has been made it may be that several species are involved, including Agrobacterium luteum, which is known to occur in D. oleae [27, 29].

It was thought that perhaps the bacterial species that replaced P. savastanoi under our culture conditions was necessary, but the inclusion of streptomycin in the adult diet has increased fecundity by extending longevity. Two generations have been cultured with streptomycin in the adult diet. Thus it now appears that D. oleae can be cultured without any bacteria present in the morphological sites normally occupied by Pseudomonas savastanoi.

Longevity

The longevity of D. oleae is quite variable, as shown in Figs. 5 and 6. The data shown in these graphs are based on adults reared from olives; thus the normal symbiote was present and should give a more natural picture of mortality. The female mortality shown in Fig. 5 seems unusually high for the first week as compared with the mortality occurring with 100 flies, shown in Fig. 6. The history of these two lots of flies is somewhat different with the 20 females emerging from laboratory-infested olives and the lot of 100 from field-collected puparia. However, the former flies were fed a yeast hydrolysate plus sucrose diet without choline chloride. Males tend to live longer than females.

RESULTS FROM LARVAL MEDIA

The xenic approach in trying to culture D. oleae under non-aseptic conditions, testing all the known media that have been used to culture the five trypetid species mentioned in the introduction, failed to permit larval development. These media, most of which have a carrot base, are rather fluid in nature, and D. oleae larvae would drown. The few known agar, casein and yeast media also gave poor results, as shown in Tables V and VI.

Protein

Poor results were obtained from all media tested until an enzymatic protein hydrolysate was incorporated in the media. Some larvae completed their development on media with high brewer's yeast concentrations, and where casein was included (Tables V and VI), but no oviposition was obtained from the resulting adults.

At first, yeast extract was used in combination with the protein hydrolysates, but better results were obtained with higher levels of brewer's yeast (Table VI, Fig. 8). By varying the protein source the speed of larval development was influenced as well as the size of puparia produced.

The two enzymatic protein hydrolysates that gave the best results were those of soya (Table III, Fig. 10) and casein (Table IV, Fig. 11). An enzymatic protein hydrolysate of casein permits the most rapid larval development of all the protein sources tested, nine days being the shortest period observed. An effective concentration of casein hydrolysate is about 2%. This concentration can be used in place of the 3% soya hydrolysate used in the standard

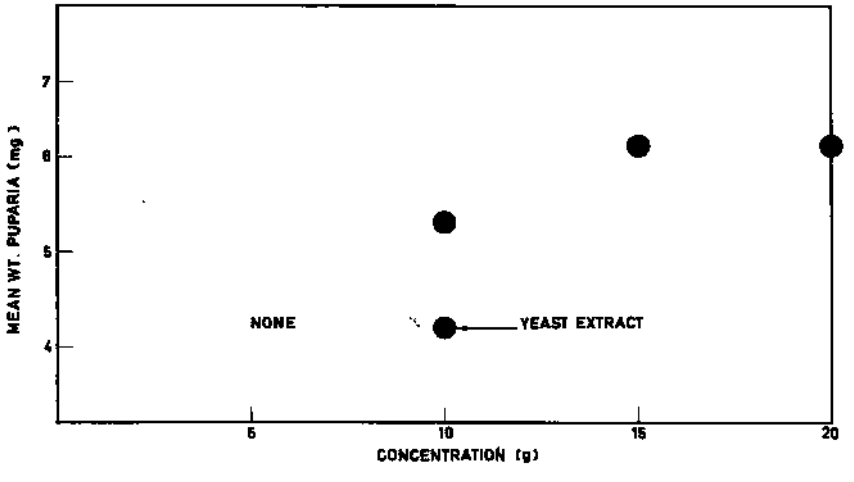

Fig. 8

Relationship between concentration of brewer's yeast
and one comparable yeast extract in the standard larval medium
and weight of Dacus oleae puparia produced
All media contained 5 g enzymatic protein hydrolysate of soya.

TABLE V

INFLUENCE OF SOME DIFFERENT CONCENTRATIONS OF BREWER'S YEAST IN BASAL MEDIA WITHOUT ADDITIONAL PROTEIN (EXCEPT FROM DEHYDRATED CARROT) UPON THE WEIGHT AND RECOVERY OF PUPARIA AND THE REPRODUCTION OF THE ADULTS PRODUCED

Brewer's yeast concn (g)	Sodium benzoate concn (g)	Citric acid (g) or HCl (ml)	No. fertile eggs	Minimum period to pupation (d)	Wt. puparia (mg)		Puparia (%)		Visible contamination	Fly emergence (%)	Production of fertile eggs
					Mean	Range	Mean	Range			
10	0.3	5								None	
15	0.3	1.5	100	18	4.1	2.0-5.9	9	5-13	Some	13	None
20	0.3	5	67	17	5.3	5.0-5.3	3	0-4	Some	50	None
20	0.35	1.5	44	16	6.2	5.5-7.5	10	6-14	Some	50	None
25	0.3	1.5	37	16	3.6	2.9-4.3	5	0-6	Heavy	None	
30	0.3	1.5	80		None				Heavy	None	
30[1]	0.3	1.0	60		None				Heavy	None	
40[2]	0.3	1.5	37	15	6.2	4.5-8.8	10	0-14	Heavy	None	

1) Without dehydrated carrot
2) With 9% dehydrated carrot instead of standard 15%.

TABLE VI

INFLUENCE OF SOME DIFFERENT PROTEIN SOURCES AND THEIR STATE OF HYDROLYSIS UPON THE DEVELOPMENT OF DACUS OLEAE LARVAE, PUPARIA AND ADULTS

Protein source	Type of hydrolysate	Concn (g)	Brewer's yeast concn (g)	Brewer's yeast extract concn (g)	No. fertile eggs	Minimum period to pupation (d)	Wt. puparia (mg)		Puparia recovered (%)		Visible contamination	Fly emergence (%)	Production of fertile eggs
							Mean	Range	Mean	Range			
Soya	Not hydrolysed	5	15	–	120		None						
Soya	" "	8	--	10	75		None						
Soya	Papain	5	--	10	185	12	3.7	2.7-5.4	33	24-45	None	90	yes
Soya	"	5	--	15	38	12	4.9	4.2-6.0	35	30-41	None	100	yes
Soya	"	8	--	10	100	12	5.2	4.3-6.6	11	10-14	None	80	yes
Soya	"	5	15	--	100	12	6.1	4.9-8.3	42	32-50	None	100	yes
Soya	"	5	20	--	50	12	6.0	5.2-7.8	55	35-62	Some	90	yes
Soya	"	8	20	--	65	12	6.0	4.9-8.1	21	16-26	Some	100	yes
Soya	"	10	20	--	30	12	6.7	5.6-7.7	44	35-62	Some	100	yes
Soya	Trypsin	5	--	10	50	16	3.7	2.9-4.9	18	18-18	Heavy	70	None
Soya + Casein	Papain Trypsin	5	15	--	50	13	5.3	4.0-7.0	22	0-44	None to Heavy	100	yes
Casein	Not hydrolysed	8	--	10	100		None						
Casein	" "	8	--	10	55	13	4.4	3.9-4.9	3	3-3	None	50	None
Yeast	Trypsin	5	15	--	100	13	5.5	4.2-7.0	19	12-33	Some	80	None
Mean	Proteose peptone	5	--	10	40	15	4.9	4.6-5.2	5	0-8	Heavy	None	

1) Other ingredients in media, such as dehydrated carrot, agar, olive oil, Tween 80, choline chloride, sodium benzoate and pH, are at the same concentrations. The temperature was 25 ± 1°C; the relative humidity was 70 ± 20%.

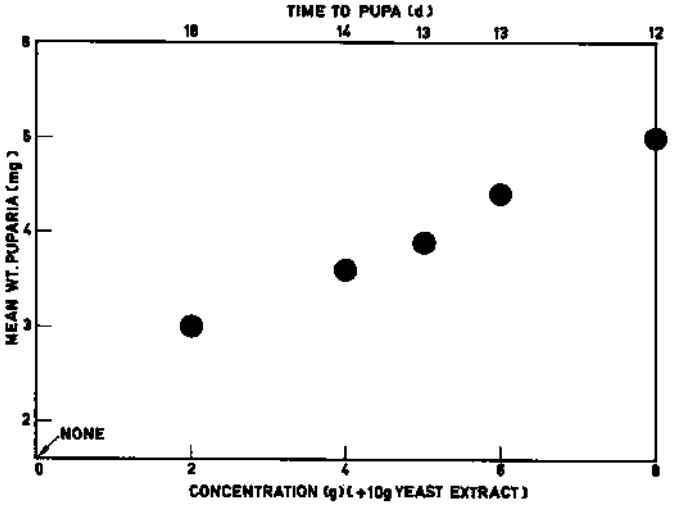

Fig. 9

Relationship between concentration of enzymatic protein hydrolysate of soya in the standard larval medium, weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage
10 g yeast extract used in place of 15 g brewer's yeast in the standard medium.

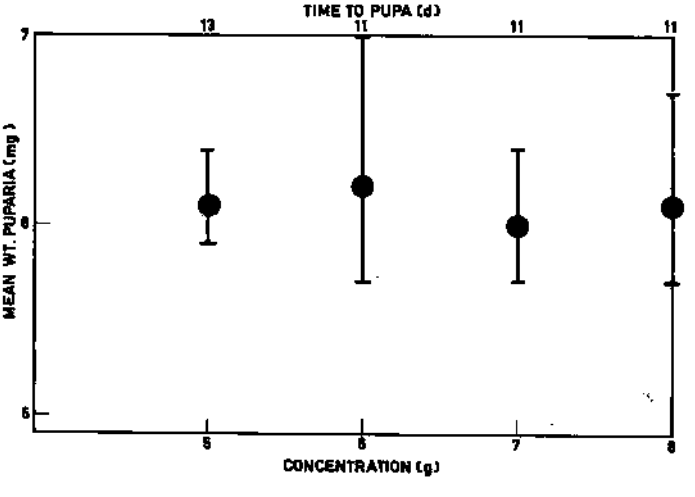

Fig. 10

Relationship between concentration of enzymatic protein hydrolysate of soya in the standard larval medium, mean weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage

diet. Enzymatic protein hydrolysates such as lactalbumin, proteose peptone, bacto-peptone and yeast gave poor results.

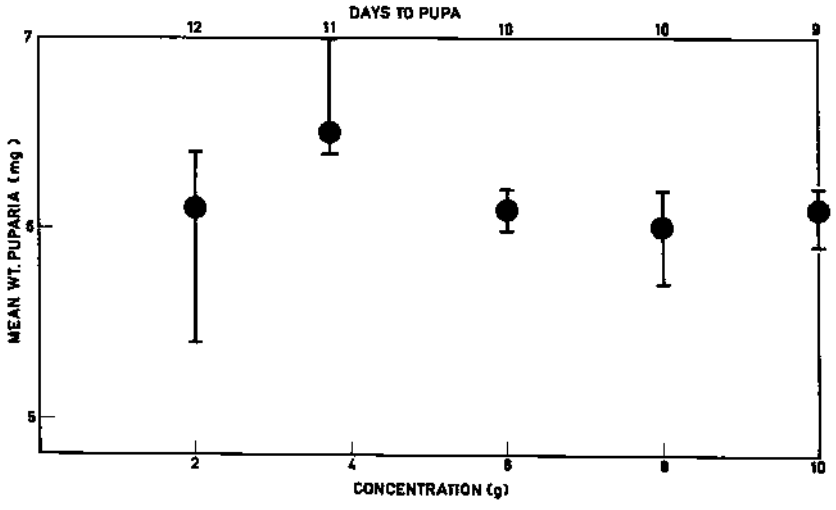

Fig. 11

Relationship between concentration of enzymatic protein hydrolysate of casein in the standard larval medium, mean weight of Dacus oleae puparia produced and minimum time required for larvae to attain pupal stage

Unhydrolysed protein sources used in the standard diet in place of hydrolysed protein such as casein, egg albumin, soya meal and dehydrated skim milk permitted none or less than 5% of the larvae to develop, and no reproduction occurred.

Carrot

Fresh carrot blended and supplemented with all the other ingredients outlined in Table II gave poor results and became rather heavily contaminated.

Dehydrated carrot from California and France*

Reduced levels of carrot permit development, but puparial emergence is reduced and adults are weak.

Brewer's yeast

Relatively high concentrations of brewer's yeast are necessary as compared with the other trypetids cultured A level of about 7% has given the best results when a protein hydrolysate is also present in the medium. Brewer's yeast is superior to baker's yeast or yeast extract, even though the biological activity of the latter is considered much higher. The water-insoluble factors present in brewer's yeast seem to be utilized, as indicated by the increased weight attained over that of yeast extract (Table VI, Fig. 8).

* The dehydrated carrot from France that was tested was donated by Dr. M. FERON, Station de zoologie, centre de Recherches Agronomiques du Sud Est, Montfaret, France.

Agar

If eggs are deposited directly into 3-4% agar which has a coating of paraffin, the eggs hatch and the larvae readily tunnel. However, in the standard medium outlined in Table II 1% is adequate. Higher levels do not seem to permit easy entry when the eggs or larvae are placed on the surface.

Olive oil

Although varying the concentration of the olive oil does not seem to influence the weight of the puparia produced or the speed of development of the larvae, the percentage of flies emerging from the puparia is influenced. About 7% olive oil permits 100% emergence and appears to allow the medium to be more extensively used. Tween 80 is necessary for emulsifying the olive oil. Concentrations above 2.5% were detrimental and Tween 40 gave inferior results.

Mould inhibitors

Sodium benzoate, n-butyl parahydroxybenzoate (Merck's Butoben), methyl paraoxybenzoate, sodium propionate and sorbic acid were tried at many different concentrations and combinations. We alternate between using the combination shown above with media containing only 350 mg sodium benzoate. Further testing is still necessary, for the media will still allow some moulds and yeasts to develop. When using protein hydrolysates it is particularly difficult to control microorganisms.

Adjusting the pH factor to about 4.1 is necessary in the attempt to control bacteria. Factors above 4.4 permits heavy contamination. It appears that one larva requires about 2 ml of the medium to develop. The cost of the larval medium is about US $1.25 per 1000, on the basis of obtaining one fly per 2 ml.

REFERENCES

[1] STEINER, L.F. and CHRISTENSON, L.D., Proc. Hawaii. Acad. Sci. Thirty-first Annual Meeting, 1955-1956 (1956) 7.
[2] CHRISTENSON, L.D., 10th International Congr. Ent. 3 (1956) 11-18.
[3] MONRO, J., Proc. Conference Tech. Use Radiation, Austral. A.E.C. (1960) 130-137.
[4] MELIS, A. and BACCETTI, B., Redia 45 (1960) 193-217.
[5] RHODE, R.H., LOPEZ, D. (F), EGUIA, F. and TELICH C.(J.), Jour. econ. Entomol. 54 (1961) 202-203.
[6] WEIDHASS, D.E., SCHMIDT, C.H. and CHAMBERLIN, W.F., Radioisotopes and Radiation in Entomology, IAEA, Vienna (1962) 257-265.
[7] Abstracts, Radiation Techniques and their Application to Insect Pests, IAEA, Vienna, Info. circ. No. 1 (1963) 14pp.
[8] STONE, W.E., "Effects of ionizing radiation on insects and other arthropods", these Proceedings.
[9] FERON, M., unpublished.
[10] LOPEZ, D. (F), Unpublished Reports, U.S.D.A. A.R.S., Ent.Res.Div. Mexico City, Mexico (1954).
[11] FINNEY, G.L., Third special report on the Oriental fruit-fly, Senate of the State of Calif. (1953) 77-83.
[12] FINNEY, G.L., Jour. Econ. Entomol. 49 (1956) 134.
[13] CHRISTENSON, L.D., MAEDA, S. and HOLLAWAY, J.R., Jour. econ. Entomol. 49 (1956) 135-136.
[14] FERON, M., DELANOUE, P. and SORIA, F., Entomophaga, 3 (1958) 45-53.

[15] MONASTERO, S. and GENDUSO, P., La lotta biologica contro la mosca della oliva, Instituto Ent. Agr. Univ. degli Studi ed Osserv. Region. per le mattie delle piante, Palermo (1962) 23pp.
[16] BATEMAN, M.A. and BIRCH, L.C., Unpublished reports, Univ. of Sydney, N.S.W.
[17] SNOWBALL, C.J., WILSON, F. and LUKINS, R.C., Austral. J. agric. Res. 13 (1962) 233-247.
[18] KORTZAS, K., Anzeiger Schädlingskunde 28 (1955) 177-179.
[19] KOLBE, W., Höfchen-Briefe No. 1 (1960) 52-89.
[20] VON BORSTEL, R.C., Science 131 (1960) 878, 880-882.
[21] VON BORSTEL, R.C., "Effects of radiation on germ cells of insects: dominant lethals, gametic inactivation and genial-cell killing", these Proceedings.
[22] THOMOU, H., "Sterilization of Dacus oleae by gamma radiation", these Proceedings.
[23] DOUGHERTY, E.C., Ann. New York Acad. Sci. 77 (1959) 27-54.
[24] MOORE, I., Ktavim 9 (1959) 295-296.
[25] MOORE, I., Entomophaga 7 (1962) 53-57.
[26] MAEDA, S., HAGEN, K.S. and FINNEY, G.L., Proc. Hawaii. Entomol. Soc. 15 (1953) 177-185.
[27] PETRI, L., Zbl. Bakter. II 26 (1910) 357-367.
[28] STAMMER, H.J., Z. Morphol. Ökol. Tiere 15 (1929) 481-523.
[29] HELLMUTH, H., Z. Morphol. Ökol. Tiere 44 (1956) 483-517.
[30] HAGEN, K.S. and FINNEY, G.L., J. econ. Entomol. 43 (1950) 735.
[31] HAGEN, K.S., Ph. D. Thesis, University of California, Berkeley (1952).
[32] HAGEN, K.S., Proc. 10th Internat. Congr. Entomol. (1956) 3 (1958) 25-30.
[33] MOORE, I. Instit. Plant Prot. Dir. Fruit Tree Entomol. Agric. Exp. Sta. Israel, Special Bull. 26 (1960) 53pp.
[34] CHRISTENSON, L.D. and FOOTE, R.H., Ann Rev. Entomol. 5 (1960) 171-190.
[35] FERON, M., Bull. Soc. Entomol. France, 65 (1960) 139-143.
[36] FERON, M., Rev. Pathol. végét. et Ent. agricol. France, XLI (1962) 3-129.
[37] ROAN, C.C., FLITTERS, N.E. and DAVIS, C.J., Ann. Entomol. Soc. Amer., 47 (1954) 593-594.

DISCUSSION

H.J. BOROUGH: Is it possible that the colour of the paraffin egg-attracting dome has any effect?

K.S. HAGEN: No specific tests have been made to determine this. Observations with a pale green colour and a paraffin colour did not seem to show much difference. Actually, the addition of 5-10% beeswax to paraffin with a melting point of 52-53°C makes the paraffin a little harder but also alters the colour slightly. The addition of beeswax appears to influence the numbers of eggs deposited. The increase in eggs may be related to colour.

G. SILVA: At what temperature did you rear your pupae? You mentioned that the rearing took nine days, but in Portugal we have never recorded less than twelve days for pupae obtained from naturally infested olives.

K.S. HAGEN: The temperature was 25 ± 1°C. I should say that the figure does not include the incubation period of the egg, which is about 2.5 d at that temperature.

M. FERON: I should like to congratulate you on the work you have accomplished in a relatively short time on such a difficult subject.

You mentioned in your oral presentation that the acidity of the culture medium prevents the development of bacteria. In general that is true, but in the course of our experiments on Dacus we found that there was some bacterial development and our microbiologist, Mr. Vago, informed us that they were acidophilic bacteria.

You also stated that the bacteria which you find replacing the symbiote

may be pathogenic for Dacus. If I remember correctly, this has also been noted in the case of Pseudomonas savastonoi.

As regards the difficulty of obtaining eggs, we made the egg-laying places more attractive to the females by adding a small quantity of olive oil to the paraffin. The females then come to lay more readily, so that the deposition of eggs on the walls of the cage is avoided, but the situation is still not entirely satisfactory and there is no doubt that the problem of obtaining and handling eggs still constitutes a great obstacle to large-scale culture.

It should be possible to determine the reproduction habits of Dacus oleae. We have devoted some time to such a behavioural study, as you know. Our studies bore particularly on sexual behaviour, but I imagine behaviour at oviposition should be largely governed by comparable conditions. The sexual behaviour involves the call of the male (vibration of the wings) followed by mating, and it is more intense at 22°C than at 25°C.

B. BACCETTI: Is it possible to say for certain that the diet of the adult Dacus contains no lipids? I should like to know this in connection with a comparative study of excretion in the larva and the adult.

K.S. HAGEN: No lipid is included in the adult diet separately. There are probably traces of sterol in the enzymatic protein hydrolysate of yeast that is used, even though it appears to be completely water-soluble. The sterol requirement in the adult can be satisfied at least partially by metabolite transfer from larval feeding. The larval medium is rich in sterols and lipids. Choline chloride supplementation in the adult food may in some way play a role in sterol or lipid metabolism.

C. PELEKASSIS: What is the effect of environmental factors, such as light intensity or relative humidity, on the oviposition rate?

K.S. HAGEN: Light intensity or photo-period do seem to have some influence on reproduction. During November and December the preoviposition period is prolonged compared with September or March. The temperature was the same in the laboratory during both periods.

M. FERON: The action of light is certainly very important in the reproduction behaviour of Dacus oleae. We got results comparable to those published for Dacus tryoni in Australia. The main factor is the duration of exposure of the adults to light. After a period of darkness corresponding to night the insects are inactive and sexual activity only commences after exposure to light for about six hours; it is facilitated by a reduction in light intensity (corresponding, under natural conditions, to dusk), but it still appears, with a certain delay, after continuous exposure to a constant light intensity. Several hours of darkness are necessary for the insect to return to a state of complete rest. The intensity of the light also plays a part. It appears that there is an optimal intensity of the order of 400 to 600 lx. These points should be investigated in further experiments.

K.S. HAGEN: What is the mode of action of the light? What is the light doing to the fly to increase this activity?

M. FERON: I think that the light acts through the medium of a hormone. With Dacus, exposure to light could result in the accumulation of a hormone required to initiate the behaviour; once a certain quantity is produced the process is triggered off, while darkness lasting for several hours might result in the elimination of the hormone.

ÉTUDE DES POPULATIONS ET DE DISPERSION DE CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) EN TUNISIE A L'AIDE DES RADIOISOTOPES

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Abstract — Résumé — Аннотация — Resumen

RADIOISOTOPE INVESTIGATION OF CERITATIS CAPITATA WIED. (DIPT. TRYPETIDAE) POPULATION AND DISTRIBUTION IN TUNISIA. Very heavy damage to Tunisian fruit production caused by Ceritatis capitata Wied. is the background to preparations to attempt control by gamma-ray sterilization of the males.

As a follow-up to ecological studies already completed and preliminary distribution tests, P^{32}-labelled flies are being used in a technique worked out for studying populations; its value seems confirmed by results obtained by conventional techniques.

The population estimate made indicates that, in different plantations, there might be 2 to 9 males per hectare by the end of winter.

ÉTUDE DES POPULATIONS ET DE DISPERSION DE CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) EN TUNISIE A L'AIDE DES RADIOISOTOPES. Les très importants dégâts provoqués par Ceratitis capitata Wied. aux productions fruitières tunisiennes sont à l'origine des travaux destinés à préparer la mise en oeuvre de la lutte par stérilisation des mâles aux rayons gamma.

En complément des études écologiques déjà réalisées et des essais préliminaires sur la dispersion, une méthode d'étude des populations a été mise au point, en utilisant des mouches marquées au ^{32}P; la valeur de cette méthode paraît être confirmée par les résultats donnés par d'autres procédés classiques.

L'estimation de population ainsi réalisée a montré qu'il pouvait y avoir de 2 à 9 mâles par hectare à la fin de l'hiver dans différentes plantations.

ИЗУЧЕНИЕ ПОПУЛЯЦИЙ И РАСПРОСТРАНЕНИЯ CERATITIS CAPITATA WIED. (DIPT. TRYPETIDAE) В ТУНИСЕ С ПОМОЩЬЮ РАДИОИЗОТОПОВ. Весьма значительный ущерб, нанесенный Ceratitis capitata Wied. плодовому производству Туниса, явился причиной проведения работ в целях разработки способов борьбы путем стерилизации мужских особей гамма-лучами.

В дополнение к уже проведенным экологическим исследованиям и предварительным опытам по вопросу о распространении был разработан метод изучения популяций с использованием мух, меченных P^{32}; представляется, что ценность этого метода подтверждается результатами некоторых других обычных способов.

Проведенная таким образом оценка популяций показала, что в конце зимы на одном гектаре различных плантаций может находиться от 2 до 9 мужских особей.

ESTUDIO MEDIANTE RADIOISÓTOPOS DE LAS POBLACIONES Y DE LA DISPERSIÓN DE LA CERATITIS CAPITATA WIED. (DIPT. TRIPÉTIDO) EN TÚNEZ. Las importantes pérdidas que la mosca Ceratitis capitata Wied. causa a la producción frutera de Túnez obligaron a emprender un estudio a fin de preparar los medios para combatirla por esterilización de los machos con rayos gamma.

Para complementar los estudios ecológicos ya realizados y los ensayos preliminares sobre la dispersión, se ha preparado un método de estudio de las poblaciones utilizando moscas marcadas con ^{32}P; el valor de este método ha sido confirmado al parecer por los resultados obtenidos con otros métodos clásicos.

El cálculo de la población así realizado muestra que a fines de invierno se encuentran probablemente de 2 a 9 machos por hectárea.

I. INTRODUCTION

L'économie fruitière de la Tunisie est commandée pour une part importante par le problème de la mouche méditerranéenne des fruits, Ceratitis

capitata Wied., qui empêche toute production fruitière d'été et oblige à de coûteux traitements sur les agrumes. C'est pourquoi l'Institut national de la recherche agronomique de Tunisie consacre un effort particulier à l'étude de ce problème.

Le Colloque d'experts sur la lutte contre les insectes par la méthode des mâles stériles, organisé par l'Agence internationale de l'énergie atomique à Vienne en octobre 1962, examinait les possibilités d'application de cette méthode à la lutte contre Ceratitis dans le bassin méditerranéen; il apparut aux membres du Colloque que la Tunisie présentait des conditions particulièrement favorables et qu'un programme d'action devait être mis en œuvre.

Le premier point du travail devait être de compléter la connaissance écologique de Ceratitis à partir des études déjà réalisées [1 - 5], ce qui a pu être entrepris avec l'aide de l'Agence.

Une prospection d'ensemble a été réalisée grâce à l'heureuse initiative et à l'aide efficace que M. Féron, Directeur de la Station de zoologie agricole de Montfavet, nous a apportées; nous l'en remercions bien vivement ici, car cela a permis d'établir les bases de ce travail écologique et de choisir une zone pilote assez favorable [6].

La connaissance aussi précise que possible de la population de mouches aux différentes époques de l'année et des possibilités de dispersion des mouches lâchées est évidemment à la base de l'application de la méthode de lutte par les lâchers de mâles stériles. C'est dans ce sens que nous avons entrepris ce travail en utilisant, conjointement avec d'autres méthodes, la technique du marquage des insectes par radioisotopes.

II. ESSAIS PRÉLIMINAIRES

En novembre 1960, en collaboration avec un expert de l'AIEA, M. J. F. Cline, un premier essai d'orientation avait été effectué dans l'orangeraie de l'INRAT: sur les 353 mouches marquées à l'aide de ^{32}P, (0,5 mc pour 100 g d'aliment à base de miel, sucre et levure de bière), lâchées au milieu des agrumes, 70, soit 20%, ont été reprises grâce à des gobe-mouches disposés à cet effet et appâtés avec du phosphate bi-ammonique à 2%. La distance maximum, parcourue en trois jours, avait été de 315 m.

L'année suivante, au milieu d'un important verger de pêchers en pleine maturité, 2200 mâles de Ceratitis étaient lâchés. Un réseau de 280 pièges au phosphate bi-ammonique permettait de reprendre 449 mouches, soit 20,4%; la moitié de ces mouches étaient capturées à moins de 100 m du point du lâcher, un quart entre 100 et 200 m et un quart au delà jusqu'à un maximum de 610 m (malgré la présence de haies brise-vents). La rapidité de dispersion atteignit 460 m en 24 h [7].

L'intéressant taux de recapture observé au cours des essais préliminaires et la facilité d'emploi du ^{32}P pour le marquage des mouches nous engageait à poursuivre les travaux entrepris.

De ce fait, un programme fut mis sur pied en 1962, destiné à fournir les renseignements de base nécessaires à une future éradication de Ceratitis en Tunisie, utilisant la méthode de la stérilisation des mâles à l'aide des rayons gamma.

Ce programme a pu entrer en action grâce à l'AIEA qui a apporté une contribution financière importante et la compétence d'un de ses experts, M. Jurenka, qui nous a utilement guidé dans la mise au point de la technique de marquage.

III. TECHNIQUE DE MARQUAGE ET DE LÂCHER

Afin de préciser la forme de ^{32}P et les doses à adopter pour notre étude, un marquage au laboratoire a été expérimenté, permettant de comparer la forme acide PO_4H_3, la forme neutre PO_4HNa_2 et le phosphore rouge irradié (91 h d'irradiation).

Le phosphore rouge fut tout de suite écarté, du fait qu'il se présentait en poudre très peu soluble.

Des formes acide et neutre, la première fut choisie pour la suite des travaux en raison de sa bonne efficacité et de son prix moins élevé; la concentration fut fixée à 1 mc pour 100 g d'eau sucrée à 10%.

Le type de boîte de lâcher, mis au point au laboratoire et qui s'est révélé à l'expérience être fort bien adapté à notre travail, permet de contaminer 100-200 mouches à la fois; à cet effet, 20 cc de solution radioactive imbibent une éponge artificielle disposée au fond de la boîte. Celle-ci peut être accrochée à une branche et son couvercle levé à distance grâce à un long fil de nylon, ceci pour éviter que les mouches contaminées ne viennent aussitôt au contact de l'épiderme (suivant une réaction assez fréquente).

IV. ESTIMATION DE POPULATION ET DE DISPERSION DANS UNE ORANGERAIE

Un essai fut réalisé dans une orangeraie proche du laboratoire pour tester l'efficacité de la technique et si possible pour en tirer quelques indications sur la présence de Ceratitis à la fin d'un hiver particulièrement rigoureux.

Deux mille mouches, en 10 boîtes de lâcher de 200 mouches, à raison de 1 boîte pour 4 arbres, furent lâchées à la fin mars de cette année au centre d'une orangeraie de 8 ha. Le temps était incertain, avec beaucoup de vent, et la pluie se déclencha au moment où étaient libérées les dernières mouches. La pluie, assez forte, dura 1 ha. Puis un vent soutenu assécha le sol sablonneux.

Les boîtes de lâcher, laissées ouvertes pendant 6 h, ne contenaient plus au total que 127 mouches mortes ou inactives. Près de 400 pièges en plastique étaient alors disposés sur toute l'orangeraie, à raison de 1 pour 4 arbres; l'attractif utilisé était le medlure, suivant la méthode américaine [8].

Le lendemain, 28 de ces pièges avaient repris 125 mouches, toutes fortement contaminées. La dispersion, avec le mauvais temps, s'avéra très faible. Trois jours après, 126 autres mouches étaient reprises, dont 2 non marquées.

Le taux de recapture a donc été de 13,3%; si l'on compte les 2 mouches non marquées comme étant de la population naturelle, cela nous permet

d'estimer, d'après l'indice de Lincoln [9], cette population naturelle comme étant de 15 mâles pour les 8 ha de l'orangeraie, soit peut-être une trentaine de mouches mâles et femelles, si le sex-ratio est de 1; c'est-à-dire 4 mouches par hectare.

V. ÉTUDE DE POPULATION ET DE DISPERSION DANS UNE ZONE PILOTE - PREMIERS RÉSULTATS

La zone pilote choisie pour les premiers essais de lutte par lâchers de mâles stériles présente des caractéristiques géographiques fort intéressantes. En effet, cette zone est isolée entre la mer et une région de collines arides s'élevant de 200 à 300 m. Elle comprend des vergers composés d'essences diverses constituant un foyer permanent typique pour Ceratitis.

Les vergers choisis pour l'expérimentation comprennent surtout des abricotiers et des figuiers, hôtes de choix de la Trypétide. Des brise-vents cloisonnent assez étroitement les parcelles, offrant à la mouche un biotope très favorable.

Un premier lâcher a été réalisé au début d'avril. Les mouches marquées ont été lâchées dans un secteur de 1 ha environ, leur nombre étant calculé à raison de 1 pour 5 m^2 de terrain, compte tenu d'une mortalité estimée à 6% d'après les précédents essais. Ces mouches, au total 1843, étaient réparties dans 19 boîtes placées dans 11 parcelles du secteur.

Le temps étant ensoleillé, les mouches sortaient rapidement des boîtes (la mortalité fut de 7,6%).

Le dispositif de recapture comprenait 300 pièges secs à raison de 1 par arbre sur la totalité du secteur (1 ha). Les recaptures ont été en 24 h de 425 mouches, soit 23% plus seulement 2 non marquées. Ceci permet d'estimer la population naturelle à 9 mâles environ par hectare (soit peut-être une vingtaine de mouches mâles et femelles).

Ce chiffre faible serait normal à la fin de l'hiver; il est confirmé d'une part par l'essai effectué deux semaines auparavant à l'orangeraie de l'INRAT, même si ce verger se trouve à 50 km de la zone pilote; d'autre part, l'étude de population en cours dans la zone pilote est contrôlée par d'autres recherches, conduites par des méthodes différentes:

En effet, depuis janvier 1963, une centaine de gobe-mouches appâtés aux protéolysats à la dose de 7% ont été installés sur diverses espèces fruitières, assez éloignées de la zone du lâcher; or, en 3 mois de relevés hebdomadaires, seules 3 mouches ont été capturées;

D'autre part, des traitements de choc appliqués mensuellement sur 4 bigaradiers (production d'hiver), 4 abricotiers (production de printemps) et 4 figuiers(production d'été) n'ont encore pas révélé la présence de Ceratitis au cours de l'hiver; il est vrai qu'il ne s'agit que de 12 arbres, alors que le dispositif de recapture des mouches marquées comprend 300 arbres, chacun muni d'un piège;

En hiver également, le ramassage des fruits tombés sous 10 arbres fruitiers, hôtes de Ceratitis à cette époque (brigaradiers), a seul permis de révéler une infestation. Les fruits ramassés sous les 10 bigaradiers,

puis placés en observation en laboratoire, chauffé en hiver, ont donné jusqu'à la mi-mars, 146 larves.

Par ailleurs, le pouvoir de dispersion des mouches marquées à partir du point de lâcher a pu faire l'objet de deux contrôles.

a) D'une part, les résultats du piégeage dans les 300 arbres de la zone de lâcher ont montré une répartition assez remarquablement homogène des captures par arbre; les mouches, en effet, se répartissent après quelques heures sur l'étendue de chacune des parcelles.

b) D'autre part, des barrages constitués par 180 pièges secs ont été disposés à des distances de 500 à 1000 m autour de la zone de lâcher 2 jours après le lâcher et laissés en place pendant 3 jours. Dans trois de ces pièges seulement, situés à environ 700 m du lâcher, 4 Ceratitis mâles ont été capturés; examinés au G-M, ils présentaient une contamination faible, mais nette (3 fois le bruit de fond); il s'agissait donc de mouches marquées.

VI. CONCLUSION

Cette première série d'opérations a permis de mettre au point les techniques de marquage, de lâcher et de capture de Ceratitis dans les conditions rencontrées en Tunisie. Les résultats obtenus dans des biotopes différents, contrôlés par deux autres méthodes, montrent entre eux des similitudes qui confirment la valeur de la méthode du marquage radioactif pour des études de population et de dispersion.

D'un point de vue plus immédiat, il apparaît que la population de mouches, au cours de l'hiver 1963 dans des foyers permanents de Ceratitis, se situe à un niveau faible qui serait de l'ordre de 2 à 9 mâles par hectare. Cette dernière donnée est fondamentale si l'on veut envisager l'application de la méthode de lutte par des lâchers de mâles stériles.

Il est évident que ces premiers résultats n'ont qu'une valeur indicative et qu'ils constituent le début d'un travail écologique qui sera poursuivi tout au long de l'année de façon à bien connaître ces variations de population de Ceratitis dans les foyers permanents.

RÉFÉRENCES

[1] DELANOUE, P. et SORIA, F., « Les fruits de l'oranger amer (Citrus bigaradia Risso), réserve dangereuse en Tunisie de mouches des fruits (Ceratitis capitata Wied.) », Ann. INRAT (1963) (à paraître).

[2] SORIA, F., « Evolution des chutes de fruits dans un verger d'agrumes en Tunisie, au cours de la saison agrumicole 1960-61 », Ann. INRAT 32, 95-108, 1959 (1961).

[3] SORIA, F., « Plantes-hôtes secondaires de Ceratitis capitata Wied. en Tunisie », Ann. INRAT 35, 51-72 (1962).

[4] SORIA, F., Epoques d'infestation de divers hôtes de Ceratitis capitata Wied. dans le Nord de la Tunisie. Rapport sur les travaux de recherche effectués en 1961 à l'INRAT (1962).

[5] SORIA, F., Evolution des infestations de Ceratitis sur les fruits de Tunisie (1963) (à paraître).

[6] FÉRON, M., La lutte contre la mouche méditerranéenne des fruits, Ceratitis capitata Wied., en Tunisie. Possibilités d'application de la méthode des lâchers de mâles stériles. Rapport de mission (1-22 décembre 1962).

[7] SORIA, F. et CLINE, J. F., « Etude du vagabondage de Ceratitis capitata Wied. en Tunisie à l'aide de radioisotopes », Ann. INRAT 32, 79-94, 1959 (1962).

[8] Auteurs divers, Mediterranean Fruit Fly (Ceratitis capitata Wied.). Survey. USDA, Document n° 803, 17 (2 juin 1959).

[9] ALLEE, W. C., EMERSON, A. E. et al., Principles of animal ecology, Saunders & Co, Ed., Londres (1949) 1-837.

DISCUSSION

M. FERON: The work done in Tunisia by Mr. Soria is really very interesting and important. Some of the best experts in the sterile-male technique attended the meeting the Agency organized in Vienna last October*, and I remember in particular that Dr. Lindquist, who is responsible for its application against the screw-worm, laid great emphasis on the need for carrying out very detailed ecological studies beforehand. Dr. Lindquist recalled that in the case of the screw-worm such studies had started before the War and an extremely accurate ecological study had a vital part in the success of the operation. He pointed out that, in general, the real number of insects in a given biotope was inadequately known and entomologists could expect surprises, but usually agreeable ones, i.e. there were far fewer insects than was expected. Observations are, of course, usually made in places where the insects accumulate. Ceratitis can be found in large numbers in an apricot plantation when the fruit is ripening, but then there will probably be few in neighbouring plantations where no such fruit is to be found. This is very important to know, as the vital information is the number of insects per hectare in a given area.

The pilot zone study referred to by Mr. Soria should therefore be continued, certainly for a year, and very probably for a second year, before it can be exactly known where the sterile males should be released. To devise a satisfactory method of releasing, tests can of course be made in smaller areas.

I have one comment on Mr. Soria's results. It is obviously taking a risk to extrapolate a population estimate on a figure of 2 non-labelled flies out of 425 recaptured. There are fortunately complementary methods which validate these figures and show that the real population is extremely small, so that the figures of 2 to 9 males per hectare are no doubt correct. What is important is to have some means of following the population gradient which takes place in about May, and the regression which takes place in autumn.

Another difficulty in estimating the total population is the real sex ratio, the real proportion of males to females. Your calculation assumes that they are equal in number, but this is very difficult to confirm. We know from breeding experience that the males of Ceratitis are more delicate and stand up less well to unfavourable conditions. The same may be true under natural conditions. Here, what we are interested in is the actual number of males, since our aim is to apply the sterile-male technique; the female population is less important, the whole point being that there should be more sterile males than normal males.

You have put one trap per tree throughout the release area; I think one could afford to be more economical in future. Your results show that the

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* Panel on insect population control by the sterile-male technique.

flies spread evenly over the whole of the test area in a few hours, so that a statistically evaluated sampling procedure should be sufficient. This should make it possible to simplify your arrangements and so facilitate the ecological work.

There is one unknown factor which you are very well aware of, i.e. you do not know the duration of life of the males released. For example, if they live longer than a month and you make monthly population studies, your conclusions may be thrown out.

F. SORIA: This will be seen at the next release.

M. FERON: That is quite true. Another aspect of the sterile-male programme which must be considered at the same time is that of devising irradiation methods in order to obtain very large numbers of sterile males. Fairly accurate information is already available from the work done by the Americans in Hawaii and this provides an order of magnitude, but it must be adapted to our conditions and to the biological material we are dealing with; their insect strains are necessarily different from what we have in the Mediterranean, and all the dosimetry and biology work must certainly be checked before we can safely release both males and the females which it would be dangerous to release if they also were not rendered sterile.

There is one remark I should like to make on our own recent work. We unfortunately did not find a method of separating females from males before the insects hatched out, and we tried to find if there was a difference at the pupal stage. There is in fact a difference in weight but also unfortunately a difference in volume. On the average the female pupae weigh 0.5 mg more than the males. This is very little and the weight graphs for the two sexes are so similar that no automatic mechanical way of separating the pupae can be seen at the moment. We also made some experiments with the eggs and were no more successful; we could not find a difference in the relative density of the eggs containing males and females.

Hence if they are to be separated, attractants must be used; otherwise the risk must be taken of releasing females at the same time as males, having first made absolutely sure that the females are sterile.

F. SORIA: I should like to thank Mr. Feron for his advice and very welcome suggestions. Now that the work has become routine in Tunisia we must begin to plan the follow-up. It would be impossible to go directly from the ecological study to the stage of making releases without knowing how the Tunisian flies will react to gamma-ray treatment. Accordingly a great deal of laboratory work must first be done. Once the doses have been determined small releases can be made in limited areas - a small-holding, an isolated orchard in a cereals area, or one of our many oases.