CHEMOSTERILIZATION AND REPRODUCTIVE PHYSIOLOGY

(Session IV)
EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON REPRODUCTIVE CAPABILITIES

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Abstract

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The use of chemotherapeutic agents in medicine has raised concerns about their potential effects on reproductive capabilities. This research aims to explore the impact of these agents on reproductive health and fertility. It is crucial to understand the mechanisms by which chemotherapeutic agents might affect reproductive outcomes, as this knowledge can inform safer treatment protocols for individuals requiring chemotherapy. The study will focus on evaluating the effects of various chemotherapeutic agents on reproductive endpoints such as fertility, embryonic development, and neonatal survival. The results will provide valuable insights into the potential reproductive risks associated with chemotherapy and inform future research and clinical practices.
Survey paper

EFFECTS OF CHEMOSTERILANTS ON REPRODUCTIVE ORGANS AND EMBRYOGENESIS IN INSECTS

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Abstract

EFFECTS OF CHEMOSTERILANTS ON REPRODUCTIVE ORGANS AND EMBRYOGENESIS IN INSECTS.

The use of chemosterilants in practice is limited by insufficient choice of suitable compounds which would be non-toxic or mutagenic to vertebrates. In testing new compounds data on the effect of chemosterilants on reproductive organs and embryogenesis is required. The present knowledge of the following topics is discussed: (1) the effects of chemosterilants on ovaries and oogenesis, (2) the effects of chemosterilants on testes and spermatogenesis, (3) the effects of chemosterilants on embryogenesis, (4) fertility effects of compounds with juvenile hormone activity. Chemosterilants affect in specific ways parastigmatic, telotrophic and polytrophic ovaries. With males in addition to the induction of dominant lethal events, greater attention should be given to the changes in spermatogenesis itself, to the effect on the secretion of accessory glands and to the activation of sperm. The embryology is affected in certain well defined ways: in the initial phase of development of the embryo in cleavage division (alkylating agents), in advanced organogenesis (antimetabolites), or before hatching (fumigants). Compounds with juvenile hormone activity as chemosterilants are promising. They are directly transferred by copulation and affect the embryogenesis in a specific way. Non-segmented, apodal, asymmetric and miniature embryos are formed. No autolysis takes place; the deformed embryos survive for quite a long time.

INTRODUCTION

Thanks to comprehensive publications (Boškovec, 1966; La Brecque & Smith, 1968) and general and special reviews (in recent years e.g. Boškovec, 1968; Knipling et al., 1968; Ascher, 1969) we have quite a lot of information on chemosterilization as well as the research accomplished. Nevertheless, chemosterilants have not been employed to a desirable extent, particularly where their prospects are most promising, i.e. in long-term autochemosterilization in natural populations. This is especially due to the still insufficient choice of suitable compounds, i.e. compounds which would have specific effects, would not be toxic and would have no mutagenic or carcinogenetic effects on other animals, in particular vertebrates.

The following aspects give a quite reli able picture of the effects of individual compounds in routine screening: fertility, hatchability, pupation, and perhaps also the emergence of adults and their sex ratio. But if we are to progress efficiently in seeking new chemosterilants and to be able to consider applying them in practice, we have to know more about their effects on the reproductive organs and on the development of individual insect species.
In this paper we shall attempt to present a survey of present knowledge in this field. We have based it on the excellent review given by LaChance et al. (1968) and on the report and critical appreciation of the problem by Saxena (1969). We make use of data from publications dealing with the topic as well as results obtained in the Institute of Entomology, Czechoslovak Academy of Sciences. The paper is divided into the following sections: 1. Effects of chemosterilants on ovaries and oogenesis; 2. Effects of chemosterilants on testes and spermatogenesis; 3. Effects of chemosterilants on embryogenesis; 4. Sterilizing effects of compounds with juvenile hormone activity.

1. EFFECTS OF CHEMOSTERILANTS ON OVARIIES AND OOCYGENESIS

Most of the research has been done on flies (Musca domestica L., Cochliomyia hominivorax (Coquerel), Drosophila melanogaster Meigen, Aedes aegypti (L.) and others), on species with polytypic, most perfectly evolved ovarioles. Destruction of egg chambers and changes taking place during their degeneration in Musca domestica after treatment with tepa, metepa and apholate have been described by Morgan & LaBrecque (1962, 1964 a, b), after hempe by Morgan (1967), after tretamine, methylimethanesulfonate and hydroxyurea by Kissam, Wilson & Hays (1967), after thiophane, metepa, tepa and apholate by Combiseco et al. (1968), after p, p-bis (1-aziridinyl)-N-methylphosphinic amide and p, p-bis (1-aziridinyl)-N-(3-methoxypropyl) phosphinothionic amide by Wilson & Hays (1968). Complete degeneration of egg chambers through irregular clusters of chromatin has been observed in Drosophila melanogaster after treatment with apholate by Cantwell & Henneberry (1963), in Anopheles Van Thiel after tepa by D’Alessandro et al. (1963). LaChance & Leverich (1968a) found that in Cochliomyia hominivorax alkylating agents affect most the endodermis of nutritive cells, which takes place immediately after emergence. Thus all the authors have observed that the main impact is directed on egg chambers during vitellogenesis, and a slightly weaker effect on the germarium.

The effects of several tens of compounds from the groups of alkylating agents, antimetabolites, steroids, and other chemicals on Musca domestica have been studied in the Institute of Entomology of the Czechoslovak Academy of Sciences. Some of the results have been published (Landa & Řežábová, 1965; Řežábová & Landa, 1967; Řežábová & Landa, 1968; Bennett-Řežábová et al., 1968; Landa, 1970; Matofín & Landa, 1970).

Changes induced by a dose of 4 - 8 µg of metepa can serve as an example. The division of oogonia in the germarium is affected in the upper zone. In the bottom one it prevents oocytes and trophocytes from growing and, in particular, it inhibits multiplication of follicular cells and formation of the follicular sheath of the third egg chamber. The main effect is specifically directed on the egg chambers, including also the first, oldest one. If metepa is applied before the vitellogenesis, it affects the endodermis of trophocytes. However, it attacks most heavily the follicular epithelium. Nucleoli and then nuclei duplicate and a rapid tumor-producing proliferation takes place. A quick degeneration follows, during which the contents of the egg chamber are resorbed. The changes are also anatomically apparent. The chambers become milky, opaque, of an irregular shape, domed have a somewhat membraneous of the.

The changes in the biochemically and functional of the tumour is extensive and by an increase is suppressed, activation of precursors of, and the activity of pro.

The study of mitic egg chambers has an applied. Oxygen content (stage of vitellogenic) show a higher metabolism in animals on the egg (Řežábová & Turner, 1970).

No detailed data on panois and panois ovaries results obtained in the of Sciences.

Changes in telotrophic metepa and apholate in Acanthoscelides obtectus et al. 1968; Řendák; the upper zone of the has been blocked. The nutrition was limited but no influence was constant. The grow and nuclei of the oocytes develop in their sex and connected with other cells, or their nuclei, oocytes are not evenly separated; the previle to the lack of follicular degenerate. If the vitellogenesis, it is generally occurs with the first egg. After a certain time, similar results were obtained.

Changes in panois in the firebrat Thermobia myrmecomorpha affects all dividing cell protocaryogenesis. Alre inhibited; transitory-staging young oocytes appear nuclei of the protofollicle taking place. In the progress changes leading to the development integration and, finally;
of an irregular shape, and they burst easily. The steroids and thalidomide have a somewhat different effect — after their application the cellular membranes of the follicular epithelium are decomposed.

The changes in the follicular epithelium have been studied also biochemically and from the viewpoint of metabolic changes. The formation of the tumour is accompanied by a multiple growth of the DNA contents and by an increase in the contents of RNA. The protosynthesis is suppressed. Autoradiographic studies confirm an increased incorporation of precursors of the nucleic acids before the proliferation begins, and the activity of proliferating nuclei (Režárová, 1968).

The study of mitochondria isolated from normal as well as treated egg chambers has shown the specific effects of the chemosterilants applied. Oxygen consumption increases, depending on the phase of development (stage of vitellogenesis). Mitochondria isolated from treated ovaries show a higher metabolic activity, even before the effect of the chemosterilants on the egg chambers appears in their morphology (Bennett-Režárová & Turner, 1970).

No detailed data on the effects of chemosterilants on telotrophic and panoistic ovaries have been published. We shall therefore use results obtained in the Institute of Entomology of the Czechoslovak Academy of Sciences.

Changes in telotrophic ovaries after the administration of metepa, metepa and apholate were studied in the beetles Hylobius abietis (L.), Acanthoscelides obtectus Say and Trogoderma granarium Everts (Landa et al., 1968; Ondráček & Mathis, 1970). The chemosterilants affected the upper zone of the germarium where the division of trophocytes had been blocked. The number of nuclei in the third zone decreased. Secretion was limited but not blocked; the core remained.

The influence was concentrated on the fourth zone, where young oocytes grow and nuclei of the prefolicular cells are divided. The primary oocytes develop irregularly, but yet they grow, partly at least, because they are connected with the trophic core. However, the prefolicular cells, or their nuclei, are entirely decimated, so that the descending oocytes are not enveloped by the follicular epithelium and are not separated; the previtellarium and vitellarium do not grow longer owing to the lack of follicular cells; and the oocytes are compressed and finally degenerate. If the vitellogenesis has already begun at the time of application, it is generally completed; if not, resorption takes place, beginning with the first egg. After only one application regeneration may occur after a certain time, and the development continues with some anomalies. Similar results were obtained on Pyrrhocoris apterus, where the formation of compound eggs was experimentally induced with 6-azauridine.

Changes in panoistic ovaries due to the effect of metepa were studied in the firebrat Thermobia domestica (Pack.). Metepa quickly and intensely affects all dividing cells as well as the nuclei of cells with increased protosynthesis. Already on the third day the division of oogonia is inhibited; transitional stages of young oocytes perish and are resorbed. Degenerating young oocytes appear in the germarium as white droplets. Also the nuclei of the prefolicular cells stop dividing and degenerative pynosis takes place. In the previtellarium the nuclei of primary cells undergo changes leading to their dissolution, vacuolisation of cytoplasm, dis-integration and, finally, to the resorption of the whole previtellarium.
The total effect of melepa on the ovarioles appears within 7 - 10 days in the reduction of the germarium to a mere 'casp' with a few cells, and in the shortening or complete resorption of the previtelarium and vitel- larium. The application does not affect molting at all. One dose is enough to block the development of ovarioles for about 40 days (three to four instars). Afterwards a partial regeneration takes place. Of course the development is very irregular.

The study of the effects of chemosterilants on different types of ovarioles provides some opportunity of discovering the principles of activity of individual compounds. Also the application of these compounds on resistant strains is likely to produce promising new information (Abasa, 1968; Turner & Maheswary, 1969). The effect of chemosterilants on the endocrine system will require special attention; the proportion of their direct and indirect effects will have to be ascertained.

2. EFFECTS OF CHEMOSTERILANTS ON TESTES AND SPERMATOGENESIS

Our knowledge of the effect of chemosterilants on testes and spermato- genesis is much less complete than on ovaries and oogenesis. The classical sterile-male technique is based on the sterilization of males by irradiation, which causes the rise of dominant lethal mutations. The earliest work on chemosterilants was also aimed primarily in this direction, and the most extensive studies on the effect of chemosterilants on spermatogenesis were mainly concerned with dominant lethal events. Mutagens affect either entire chromosomes or individual chromatids. Changes are not usually found on sperm, but most often they are evident in anomalies in the division of the zygote in the fertilized egg. Dominant lethal mutations in Cochliomyia hominivorax were described in detail by LaChance & Riemann (1964) and LaChance & Crystal (1965). Information on and problems connected with dominant lethal mutations produced in insects by irradiation as well as sterilants have been presented by LaChance (1967), who includes a detailed bibliography comprising also works which dealt with this topic long before the era of chemosterilants.

Of course chemosterilants affect testes as well as spermatogenesis and the vitality of ripe sperm. Because the practical application of strong mutagens is out of the question, the possibilities of using aspermia or inactivation of sperm for sterilization have lately been considered. In general, we can say that spermatogenesis is affected in a lesser degree than oogenesis. This conclusion is connected also with the fact that in females chemosterilants have a marked effect on that phase of oogenesis which has no counterpart in males. Complete aspermia is an extreme manifestation of the influence of chemosterilants on spermatogenesis, but it does not occur very often. It follows from papers published so far (Cantwell & Henneberry, 1963 - apholate on Drosophila melanogaster; Keiser et al. 1965 - eight compounds on Dacus cucurbitae Coquillett; Dacus dorsalis Hendel, Ceratitis capitata (Wiedemann)) that spermatogonia undergoing division are affected the most; somewhat less affected are young spermatoocytes, and least of all the stages of spermateliosis. With the destruction of cells the whole testes become smaller; this has often been reported (Lindquist et al., 1964 - apholate on Anthometrus grandis Boheman; St Loew). In contrast, 1964 - apholate on A. on Diparopsis castan be fully developed; its greatly varies with the sterility can cause a Hamilton & Sutie. glands in Diabrotica glands degenerate an. Seeing that the filling to vitellogenesis in fee line of research.

The production of sterilants on sperma sperm, which may le & von Borstel, 1954: Ashmead; LaChance Habrobracon jaglandi et al. (1966) studied Musca domestica for found that the degree motility of sperm. They will require further c. a complicated process melolontha (L.)).

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3. EFFECTS OF CI

Detailed data on were obtained from t. vorax and Habrobracon. Embryogenesis i of the embryo is usu dominant lethal muta lethal mutations have & von Borstel, 1954; dominant lethal muta by the male (sperm) & Henshaw, 1941). 2: at the early stage of derm (LaChance & R
in 7 - 10 days in new cells, and in water, sodium bicarbonate, and vitellogenin. One dose is equal to 10 days (three doses) in place. Of the different types of compounds, one type contains the principle of spermatogenesis. The information obtained on the proportion of chemosterilants and the proportion of sterility can be used for studies on the effects of chemosterilants on spermatogenesis and the degree of sterility.

In summary, the use of chemosterilants in the field of spermatogenesis is often followed by lowered vitality of sperm, which may lead to its immotility or even mortality. (Whittington & von Borstel, 1964 - nitrogen mustard on Habrobracon juglandis Ashmead; LaChance & Leverich, 1968 - nine chemosterilants on Habrobracon juglandis). Acher & Avdat (1967) and especially Acher et al. (1968) studied the degree of motility of sperm in spermathecae of Musca domestica females sterilized with Brinton and Tinicide. They found that the degree of fertility is in direct relation to the degree of motility of sperm. The activation of sperm is a complex process that requires further detailed study (Landa, 1960), for example, observed a complicated process of activation in the spermatheca of Melolontha melolontha (L.).

Our preliminary study of the effects of chemosterilants on male Melolontha melolontha has generally confirmed the published results. Metepa, 5-azauridine and especially hempa applied in the course of spermatogenesis inhibited the division of spermatocytes and cause their degeneration. Spermatozoa are not morphologically affected. An extensive study of the effect of chemosterilants on spermatogenesis, on the secretion of accessory glands and the activation of sperm has been launched. The objects are Musca domestica, Melolontha melolontha, Apis mellifera L. and Laspeyresia pomonella (L.).

3. EFFECTS OF CHEMOSTERILANTS ON EMBRYOGENESIS

Detailed data on the effects of chemosterilants on embryogenesis were obtained from the study of Musca domestica, Cochliomyia hominivorax and Habrobracon juglandis.

Embryogenesis is the process most affected and the development of the embryo is usually inhibited in the initial phase. This is due to dominant lethal mutations causing the death of the syngote. Dominant lethal mutations have been induced by a number of compounds (Whittington & von Borstel, 1954; von Borstel, 1955; Atwood et al., 1954). The dominant lethal mutation causing sterility may be brought into the syngote by the male (sperm) as well as female (nucleus of the oocyte) (Sonnenblick & Heneshaw, 1941). The development of the embryo is most often inhibited at the early stage of a few cleavage nuclei before the formation of blastoderm (LaChance & Riemann, 1954; LaChance & Crystal, 1955; Smittle,
An extraordinary methyl-7,11-dihydropyrazolone has been discovered as a compound that administers sterility throughout the life of the treated male and when mating with females. This effect is inhibitory to the embryo. Non-inhibitory embryos are formed immediately after fertilization for a quite long time.

It can be seen that this effect is relatively less in generations that are free of abnormal eggs. This phenomenon is of great importance in the control of insect reproduction.

The present reiteration of the present work on these lines...

4. STERILIZING EFFECTS OF COMPOUNDS WITH JUVENILE HORMONE ACTIVITY

It has been discovered in recent years that a number of chemicals with juvenile hormone activity have sterilizing effects. These compounds have a number of excellent properties: they are not toxic, they should have no mutagenic effects, their influence is specific and they are directly transferred by copulation. Some results were published (Slama & Williams, 1966; Pyrrhocoris apterus; Riddiford & Williams, 1967; Hyalophora cecropia; Antheraea pernyi Guerin; Novak, 1969; Schistocerca gregaria Forskal), but most of the new findings are not yet in print. Compounds of this type do not inhibit the development of ovaries, rather they accelerate oogenesis. But either directly or through copulation with treated males they penetrate eggs and even in negligible doses they affect embryogenesis.
An extraordinarily powerful chemosterilizing effect of 3, 7, 11-trimethyl-7, 11-dihydrodichloro-2-dodecenoic acid on adult Pyrrhocoris apterus has been discovered (Masner et al., 1968; Matolin, 1970). The compound administered to females in a dose of 10 μg induces complete sterility throughout the reproductive cycle. If it is transferred by copulation with treated males, the percentage of sterility depends on the dose and time when males were treated before copulation (Masner et al., 1970). Embryonic development proceeds in a normal way until the blastoderm stage. It is inhibited at the time of RNA syntheses and differentiation of the embryo. Non-segmented, apodal, asymmetric and miniature embryos are formed. In contrast to what happens with alkylation agents, immediate autolysis does not take place, the deformed embryos surviving for quite a long time.

It is seen that compounds with juvenile hormone activity affect embryogenesis in a manner different from those chemosterilants mentioned in section 3. An important result was obtained by exposure of Thermobia domestica eggs to vapours of 3, 7, 11-trimethyl-7, 11-dihydrodichloro-2-dodecenoic acid. Embryogenesis was inhibited and albinotic forms were obtained. This phenomenon needs to be studied further.

The present review is certainly not exhaustive, but aims at summarizing the present techniques with a view to ensuring that future work on these lines will be effective.

REFERENCES


These compounds are directly toxic to the insects, they should be ingested by the adults (Simmon & Williams, 1967 - Hyalophora tosca, Gregaria sp. - Heliarcia). Compounds that control the development of treated males effect embryogenesis.

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coll. Int. CNRS, Tours: 411-420.


DISCUSSION

D. S. GROSCH: Do you apply the hormone to the exterior of the insect – i.e. was the method used topical application?

V. LANADA: Yes, the substance with juvenile hormone activity was applied topically.

D. S. GROSCH: What solvent do you use?

V. LANADA: The compound was used as such or in an acetone solution.

A. ECONOMOPOULOS: Do you have any information on male competitiveness or general sexual behaviour after the accessory glands have been damaged by chemosterilants?

V. LANADA: At the Institute of Entomology in Prague we have been studying the changes in the general sexual behaviour of Musca domestica L. and Pyrrhocoris apterus L., after the application of alkylating agents and compounds with juvenile hormone activity. This study was not really connected with that on the accessory glands. Some time ago we started the experiments on the accessory glands of Melolontha melolontha. The results will be published within the next year.

INTRODUCTION

The red bollworm of cotton in Central Africa is a major pest. The cotton boll is an important economic crop. The challenges include the mass-rearing of the pest and its resistance to insecticides. There are several aziridine derivatives used as insecticides. Previous work has shown that these compounds can be toxic to various insects. Further research is needed to develop effective and environmentally friendly control strategies.

STUDIES OF COMPETITIVE TREATMENTS ON BOLLWORMS

D. G. CAMPA

Tropical Pest Management

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Tropical Pest Management

Abstract

STUDIES OF COMPETITIVE TREATMENTS ON BOLLWORMS

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In a test of chemosterilants from papers soaked from male moths, we observed that the treated paper significantly reduced the oviposition rate. The results were consistent with previous studies, showing that the treated paper significantly reduced the oviposition rate. The treated paper was more effective than the untreated paper in reducing the oviposition rate. The treated paper was more effective than the untreated paper in reducing the oviposition rate. The treated paper was more effective than the untreated paper in reducing the oviposition rate.

No obvious damage to the bollworm larvae was observed.
STUDIES OF COMPETITIVENESS, CHEMOSTERILANT PERSISTENCE AND SPERM STRUCTURE IN TREATED RED BOLLWORMS, Diparopsis castanea (HMPS.)

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Abstract

STUDIES OF COMPETITIVENESS, CHEMOSTERILANT PERSISTENCE AND SPERM STRUCTURE IN TREATED RED BOLLWORMS, Diparopsis castanea (HMPS.).

In tests of chemosterilants applied by injection to adult moths Diparopsis castanea (HMPS.) obtained from pupae shipped from Malawi, the male sterility indices (LSM/SDM) for sterile, topa and metamata were 98, 19.9 and 11.8 respectively, both triphenyl and 2,4,6-trichlorophenol were lower and 1.6 and 1.7. The female sterility index of topa was 48.2. Topa treatment of female moths significantly reduced the oviposition rate. When male moths were treated, repetitive mating tests showed that topa had no significantly adverse effect on mating frequency applied at the SDG level, whereas phenoxylated treated males at such a dose level mated significantly less frequently. The sterilization effect of topa on males was permanent throughout a 5-day period, virtually the adult life-span of the moth at 25°C. Topical treatment of male moths with topa gave a sterility index of 98.6. At the SDG they were fully competitive.

A rapid rate of topa degradation occurred after injection, half-life values ranging from 14.3 h at 25°C to 18.6 h at 15°C. After topical applications, topa degradation occurred more slowly, reaching the 50% level after 45.6 h at 25°C, 72.5 h at 20°C and 143.3 h at 15°C. The results were related to the relatively slow rate of absorption from acetone solution. Potential environmental contamination hazards are considered.

No obvious damage to the ultra-structure of the sperm of Diparopsis was observed after treatment of the male moth with injected doses of topa in excess of the sterilizing level.

INTRODUCTION

The red bollworm Diparopsis castanea (HMPS.) is an important pest of cotton in Central Africa. It spends most of its larval life inside the cotton boll and is therefore particularly difficult to control by the use of insecticides. There is, however, a possibility of controlling this insect by either the mass-release of sterilized insects or by attracting the natural population to some form of bait-station.

Previous work has shown that the adult moth can be sterilized by several aziridine chemosterilants. Weak sterilizing activity was also shown by other classes of chemosterilants such as phosphoramides, s-triazines and organo-metallics [1-3]. This paper presents the results of further laboratory investigations to gain more quantitative information on the sterilizing and toxic effects of these chemosterilants having appreciable
activity against Dipsacopsis. Since the aziridines present a possible mutagenic hazard to man and higher animals, the rate of breakdown following topical and injection application of an active chemosterilant, tepa, was studied. Preliminary observations on the complex sperm ultrastructure and its possible susceptibility to damage by chemosterilants are also reported.

MATERIALS AND METHODS

Standard test procedure

The insects were obtained from pupae collected in the field and shipped from Malawi. Graded concentrations of the chemosterilants were applied to one-day-old moths of specified sex to determine the sterility and toxicity caused by known dosages. Solutions of the chemosterilants dissolved in 1 µl of distilled water or aceton were injected through the dorsal surface of the thorax or applied topically to the ventral abdominal surface, by means of a microdrop applicator fitted with an all-glass syringe equipped with No. 27 gauge hypodermic needles. Before treatment the moths were immobilized by momentary exposure to carbon dioxide.

Sterilizing effects

To determine the sterilizing effects, the insects were treated in the afternoon and mated the same evening to untreated insects of the opposite sex in disposable plastic cups (3 1/4 in. X 2 1/4 in.) partially lined with blotting paper, with one pair of moths in each cup. Plastic Petri dishes were used as lids. The cups were kept at 27°C and 70% r.h. with a 12 h photoperiod under artificial lights for 6 days. The blotting paper on which the eggs were laid was replaced each day and each batch of eggs incubated separately for 6 days at 27°C and 70% r.h. to determine whether or not sterility had been induced. At the end of the 6-day test period the females were examined for spermatophores as proof of mating and eggs from unmated females were discarded. In each replicate test 25-30 insects were treated, while a control replicate treated with solvent only was included in each test.

Mortality tests

After similar treatments of one-day-old moths, they were held in groups of 25-30 insects in plastic sandwich boxes (11 in. X 6 in. X 3 1/2 in.) for a period of 4 days under the same temperature, humidity and light conditions as the insects tested for sterility. Corrections for control mortality and sterility were made by Abbott's formula [4]. Dosage/mortality and dosage/sterility data were analysed by means of probit analysis [5]. The significance of the reduction in the rate of oviposition as a result of treatment was assessed by the Mann-Whitney U test.

Repetitive mating tests. In the repetitive mating tests, each treated male was confined individually to a mating container into which was introduced, on six successive nights, a fresh one-day-old virgin female which was removed lined with blotting paper. In each re

Permanence of was assessed by matin, and each treated mo on untreated female period of 6 days. D manner. In each re

Assay of residual days old were treated with water by injection 0 were held for vairiy 47 r.h., and 15°C with boxes (11 in. X discarded and samp high-speed homogen of tepa in the cuclu 5 min. The mixture to remove water and noted, it was evaporated. The residue was dis of chloroform extra until ready for anal Tepa was analy curve was obtained due analysis, 2-3 m tubes was evaporate added 3 ml distilled benzyl) pyridine reab oiling water bath as was added in quick s potassium carbonate. To avoid turbidity H colour intensity increa using 1-cm cells of since the blue colour values obtained were samples. Each sam recorded.

Sperm preparation made of sperm dis solution and measur leudia drawings. Fi
which was removed each morning to glass specimen-tubes (2 in. x 1 in.) lined with blotting paper and the eggs laid were assessed in the standard manner. In each replicate test 33–39 insects were treated.

Permanence of sterility. The permanence of the sterilizing effect was assessed by mating groups of 25 treated males with one- to three-day-old virgin females on six successive days post-treatment.

Competitive mating tests. The standard disposable cartons were again used as mating containers. Treatments occurred in the afternoon and each treated male was introduced in a separate carton together with one untreated female. The insects remained together for the whole test period of 6 days. Daily collections of eggs were made in the standard manner. In each replicate test 32 to 89 insects were treated.

Assay of residual tepa. Twenty-five to thirty-five male moths 2–4 days old were treated with 10 μg of tepa either in 1-μl amounts of distilled water by injection or 1-μl amounts of acetone by topical application. They were held for varying lengths of time at 27°C and 70% r.h., 20°C and 47% r.h., and 15°C and 40% r.h. under continuous light in plastic sandwich boxes (11 in. x 6 in. x 3 1/2 in.). Before extraction dead moths were discarded and samples of 25 moths were homogenized for 5 min in a high-speed homogenizer in 50 ml of chloroform. To determine the amounts of tepa in the cuticular layer, whole moths were shaken in chloroform for 5 min. The mixtures were then shaken with anhydrous sodium sulphate to remove water and then filtered. After the volume of filtrate had been noted, it was evaporated to dryness at 40–50°C in a rotary evaporator. The residue was dissolved in acetone with 1 ml of solvent for every 2 ml of chloroform extract obtained. The solution was stored in a deep-freeze until ready for analysis. Each determination was replicated three times.

Tepa was analysed by the method of Epstein et al. [8]. A standard curve was obtained for known quantities of the chemosterilant. For residue analysis, 2–3 ml of the acetone extracts contained in 10-ml calibrated tubes was evaporated to dryness in a stream of air. To each tube was added 3 ml distilled water, 1 ml pH 4 buffer and 1 ml of the γ-(4-nitrobenzyl) pyridine reagent. The mixtures were heated for 20 min in a boiling water bath and then cooled in an iced water bath. To each tube was added in quick succession 4 ml of acetone, 1 ml of 1 M aqueous potassium carbonate and distilled water to make the volume up to 10 ml. To avoid turbidity Hyflo Supercel was added and after filtration the colour intensity immediately measured at 600 mμ on a spectrophotometer using 1-cm cells of 3 ml capacity. Speed was essential at this stage since the blue colour formed was stable for only 30 min. The residue values obtained were corrected for the apparent residue found in control samples. Each sample was assayed at two dilutions and the mean value recorded.

Sperm preparations. Observations under the light microscope were made of sperm dissected from female spermathecae into insect Ringers solution and measurements of sperm length noted by means of camera lucida drawings. For transmission electron microscopy, testes were
fixed at 0-4°C for 2 h in phosphate-buffered 2.5% glutaraldehyde, post-fixed for 1-2 h with 2% buffered osmium tetroxide, dehydrated in alcohol and embedded in Araldite. Sections were taken with a Reichert ultramicrotome and double-stained with uranyl acetate and lead citrate. They were examined in a JEM 7 electron microscope. For scanning electron microscopy, squashes of fresh testes were air dried or fixed in glutaraldehyde coated in vacuo with 500 Å of gold/palladium, and examined in a Cambridge Instruments Stereoscan electron microscope.
RESULTS

Treatment of adult moths by injection: primary evaluation.

The regression lines obtained (Figs 1 and 2) showed that in all instances a linear relationship existed between the concentration of chemosterilant (log x) and the percentage sterility (probit y). The regression equations and ED₅₀ values are shown in Table I. A comparison of the heterogeneity (χ²) about the sterility and mortality regression lines fitted with and without the constraint of parallelism gave no evidence that they were not parallel in the case of tepa, apholate, metepe and hempa. This made it possible to calculate the ratio between sterilizing and toxic doses of the applied chemosterilants (Table II). This ratio will be termed the sterility index, by analogy with the term 'therapeutic index' as used in pharmacology. The regression lines for triphenyl tin acetate were not parallel so that the relative potency shown in Table II is at the ED₅₀ level only.

In males the greatest sterility index of 24.0 was shown by apholate followed by tepa with 17.8 and metepe with 11.5. For these substances, the calculated 50% sterilizing doses (SD₅₀) were 1.5, 0.73 and 2.7 μg respectively. A very low sterility index of 1.4 was obtained with hempa and a negative value of -7.7 for triphenyl tin acetate. This showed that complete sterility could only be achieved at doses with considerable toxic side-effects, a result which precludes their use as practical chemosterilants against Diparopsus.
### TABLE I. STERILITY AND MORTALITY REGRESSION EQUATIONS AND CALCULATED ED₉₀ OF CERTAIN CHEMOSTERILANTS AGAINST Diparopsis MOTHS (A) WHEN APPLIED BY INJECTION, (B) WHEN TEPA APPLIED TOPICALLY TO MALE MOTHS

<table>
<thead>
<tr>
<th>Chemosterilant</th>
<th>Sex treated</th>
<th>Sterility</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Regression equation</td>
<td>log factor</td>
</tr>
<tr>
<td>A. Tepa</td>
<td>Males</td>
<td>Y = 1.17 + 4.47x</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>Y = 4.42 + 1.42x</td>
<td>2</td>
</tr>
<tr>
<td>Apholase</td>
<td>Males</td>
<td>Y = -0.33 + 4.44x</td>
<td>10</td>
</tr>
<tr>
<td>Metepa</td>
<td>Males</td>
<td>Y = 3.86 + 2.36x</td>
<td>1</td>
</tr>
<tr>
<td>Hempa</td>
<td>Males</td>
<td>Y = -3.10 + 3.04x</td>
<td>10</td>
</tr>
<tr>
<td>Triphenyltin acetate</td>
<td>Males</td>
<td>Y = 2.76 + 1.03x</td>
<td>10</td>
</tr>
<tr>
<td>B. Tepa</td>
<td>Males</td>
<td>Y = 1.77 + 4.78x</td>
<td>1</td>
</tr>
</tbody>
</table>

A: Log sterility in sex treated (Table III). B: Mortality in sex treated (Table III). C: Toxicity in sex treated (Table III).
### TABLE II. RATIO OF STERILE AND LETHAL DOSES (as STERILITY INDEX) OF (A) CERTAIN CHEMOSTERILANTS APPLIED TO Diparopsis castanea ADULTS BY INJECTION, (B) TEPA APPLIED TOPICALLY TO MALE MOTHS

<table>
<thead>
<tr>
<th>Chemosterilant</th>
<th>Sex treated</th>
<th>Sterility index</th>
<th>95% fiducial limits</th>
<th>$\chi^2$ for parallelism of regression lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Tepe</td>
<td>Males</td>
<td>17.9</td>
<td>10.1 - 30.7</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>4.2</td>
<td>2.8 - 6.4</td>
<td>0.24</td>
</tr>
<tr>
<td>Apholate</td>
<td>Males</td>
<td>24.0</td>
<td>13.1 - 43.7</td>
<td>3.75</td>
</tr>
<tr>
<td>Metopa</td>
<td>Males</td>
<td>11.8</td>
<td>6.6 - 19.9</td>
<td>1.88</td>
</tr>
<tr>
<td>Hempa</td>
<td>Males</td>
<td>1.4</td>
<td>0.1 - 1.9</td>
<td>0.22</td>
</tr>
<tr>
<td>Triphenyl tin acetate</td>
<td>Males</td>
<td>-7.7$^a$</td>
<td></td>
<td>4.43</td>
</tr>
<tr>
<td>B. Tepe</td>
<td>Males</td>
<td>34.6</td>
<td>26.30-45.90</td>
<td>0.73</td>
</tr>
</tbody>
</table>

$^a$ For $ED_{50}$ values only.

### TABLE III. EFFECTS OF TEPA INJECTIONS ON THE MATING OF Diparopsis

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>Mating as percentage of control mating $^a$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated females x normal males $^b$</td>
<td>Treated males x normal females $^b$</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>15.6</td>
<td>24.9</td>
</tr>
<tr>
<td>20</td>
<td>75.6</td>
<td>37.5</td>
</tr>
<tr>
<td>10</td>
<td>81.0</td>
<td>83.0</td>
</tr>
<tr>
<td>5</td>
<td>48.7</td>
<td>60.0</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>106</td>
</tr>
</tbody>
</table>

$^a$ Minimal mortality at all dose levels for the 2-day mating period.

$^b$ Data based on 30 replicates at each concentration.

SD$_{50}$ for female moths 35.9 µg; SD$_{50}$ for male moths 1.9 µg.

A low sterility index of 4.2 was obtained for tepe-treated females, which again suggests that mating would be adversely affected at doses causing complete sterility. This conclusion was confirmed by mating experiments (Table III). Treatment of females with tepe also caused a significant reduction in the rate of oviposition (Table IV).
### TABLE IV. EFFECT OF TEPA TREATMENT BY INJECTION ON THE RATE OF OVIPOSITION IN Diparopsis FEMALES MATED WITH UNTREATED MALES (4-DAY OVIPOSITION PERIOD)

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>No. of mated females</th>
<th>No. of eggs</th>
<th>Mean No. of eggs per female</th>
<th>U value</th>
<th>Significance of oviposition reduction compared to control p</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>2</td>
<td>7</td>
<td>3.5</td>
<td>2</td>
<td>0.025</td>
</tr>
<tr>
<td>29</td>
<td>14</td>
<td>360</td>
<td>22.6</td>
<td>43</td>
<td>0.01</td>
</tr>
<tr>
<td>19</td>
<td>21</td>
<td>486</td>
<td>28.4</td>
<td>142</td>
<td>0.005</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>1106</td>
<td>38.2</td>
<td>229</td>
<td>0.42</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>122</td>
<td>40.6</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>18</td>
<td>558</td>
<td>41.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE V. MATING FREQUENCY AND MEAN MORTALITY OF MALE Diparopsis castanea NOTED IN REPETITIVE MATING TESTS AFTER TREATMENT BY INJECTION WITH ESTIMATED ED₅₀ STERILIZING DOSES OF TEPA AND APHOLATE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of males treated</th>
<th>Mean mortality in days</th>
<th>Mean No. of matings per male</th>
<th>Mating frequencies</th>
<th>x² values for heterogeneity for mating frequency</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>4.6</td>
<td>1.2</td>
<td>X9 X2 X2 X2</td>
<td>0.5</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TEPA</td>
<td>39</td>
<td>4.3</td>
<td>1.0</td>
<td>X9 X2 X2 X2</td>
<td>19.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apholate</td>
<td>39</td>
<td>3.1</td>
<td>0.5</td>
<td>X9 X2 X2 X2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE VI. PERMANENCE OF STERILITY OF MALES OF
Diparopsalis castanea INJECTED WITH TBPA AT ED₉₅ STERILITY
LEVEL (25 MALES PER TREATMENT)

<table>
<thead>
<tr>
<th>Time of mating</th>
<th>No. mating</th>
<th>Total eggs laid</th>
<th>No. hatching</th>
<th>% hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>in days post-treatment</td>
<td>with 1-day-old females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>1491</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>1542</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>695</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>202</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>346</td>
<td>13</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>230</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4636</td>
<td>39</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Treatment of adults by injection: evaluation of sexual vigour and permanence of the sterility effect

The relative merits of the two most suitable chemosterilants, apholate and tepa, were evaluated by repetitive mating tests. The results shown in Table V clearly indicate tepa to be the most effective chemosterilant. The mating frequency approximated closely to the control mating frequency ($\chi^2 = 0.5$), whereas the mating frequency after apholate treatment was reduced ($\chi^2 = 10$). The results show also the mating potential of the male moth. Although some insects mated three times, the mean number of control matings per male was one since many males did not mate at all.

Sterility induced in male moths by tepa injection at the SD₉₅ level was permanent. No significant recovery of fertility occurred throughout the life of the adult moth (Table VI).

Tepa treatment of adult moths by topical application

The regression lines obtained following topical application of graded concentrations of tepa again showed that a linear relationship existed between dose of chemosterilant and percentage mortality and sterility (Table III). The regression lines were parallel ($\chi^2 = 0.73$) and therefore a sterility index of 34.5 was calculated with an SD₉₅ value of 4.74 µg.

The results of competitive mating tests are shown in Table VII. The frequency of sterile mating compared with fertile mating ($\chi^2 = 3.65$) gave no evidence for significant heterogeneity and it was concluded that mating was not adversely affected as a result of treatment. A comparison of the mean fecundity of females mated with sterile males compared with those mated with fertile males ($\chi^2 = 10.93$) indicated significant heterogeneity at the 1% level. This suggested a secondary reduction in oviposition, perhaps caused by the transmission of a proportion of the chemosterilant during copulation. A comparison of the competitiveness of
<table>
<thead>
<tr>
<th>TABLE VII. COMPETITIVENESS OF MALE D. DISPAR TREATED TOPICALLY WITH 15 µG OF TEPA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of replicates</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

a In all replicates, one treated male and two normal males were included together. Matings were confirmed by dissection of females for sperm transfer at the end of the test period.

b Comparison of mean fecundity of females mated with sterile or fertile females, i.e., 10.3%, indicating significant non-homogeneity.

Table VIII. COMPETITIVE DAYS F 15 µG TEPA

<table>
<thead>
<tr>
<th>Days post-treatment</th>
<th>% sterile mating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

* Frequency of sterile mating at 5% level.

Sterile and fertile males is shown in Table VIII that some incapacity effects soon completely.

### Tepa residue analysis

The results show that and 72°C after injection of three replicates, the tepa (µg/mo) agains and by analysis of vari...From the regression of the chemosterilant has calculated.

A rapid a after all injection applied at 72°C to 18.6 h at 11 occurred much more at 72.3 h at 30°C and 14:20°C from a 10-µg topical washing of intact trit was 46.8 h.

The role of decor was lower than the rate.

### Structure of D. Dispar on the action of tepa

The mobility of sp and can readily be observed injected into males at when transferred in cc. However, at much high the spermatheca was c
TABLE VIII. COMPETITIVENESS OF MALE Diparopsis ON SUCCESSIVE DAYS FOLLOWING TOPICAL TREATMENT WITH 15 μg TEPA

<table>
<thead>
<tr>
<th>Days post-treatment when mated</th>
<th>Total matings</th>
<th>Fertile matings</th>
<th>Sterile matings</th>
<th>% Sterile matings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Expected* Actual</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>31</td>
<td>7</td>
<td>50 15.5</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>27</td>
<td>14</td>
<td>50 34.1</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>15</td>
<td>15</td>
<td>50 44.1</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>15</td>
<td>11</td>
<td>50 42.4</td>
</tr>
</tbody>
</table>

* Frequency of sterile mating compared with fertile mating, χ² = 7.81, which indicates heterogeneity at 5% level.

sterile and fertile males when mated on successive days post-treatment is shown in Table VIII. The results suggested that shortly after treatment some incapacitation of sterilized males occurred, although the insects soon completely recovered.

**Tepa residue analysis**

The results showing the varying rate of tepa degradation at 15, 20 and 27°C after injection and topical application, taking the mean of the three replicates, are given in Table IX. By plotting the log of the residual tepa (μg/moth) against time, a series of regression lines were obtained and by analysis of variance shown to be linear in all instances (Fig. 3). From the regression equations (Table X) the time in hours when 50% of the chemosterilant had been degraded after the various treatments was calculated. A rapid and very similar rate of decomposition occurred after all injection applications, the half-life values ranging from 14.3 h at 27°C to 18.6 h at 15°C. After topical application tepa decomposition occurred much more slowly, reaching the 50% level after 45.8 h at 27°C, 72.3 h at 20°C and 143.3 h at 15°C. The rate of absorption of tepa at 20°C from a 10-μg topical dose was followed by analysing a series of washings of intact treated insects. The half-life value at this temperature was 48.6 h.

The rate of decomposition of tepa applied to topically treated insects was lower than the rate of absorption (Fig. 4).

**Structure of Diparopsis sperm and some preliminary observations on the action of tepa**

The mobility of sperm is most evident after mating has taken place and can readily be observed under the light microscope. When tepa is injected into males at doses just sufficient to induce sterility the sperm when transferred in copulation to the female appears to be normally active. However, at much higher doses agglutination of transferred spermatozoa in the spermatic caeca was observed. Thus, while the primary sterilizing action
<table>
<thead>
<tr>
<th>Interval post-treatment (hours)</th>
<th>(A) Extraction of homogenized insecte</th>
<th>(B) From surface washes of whole moth after topical application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Topical application</td>
<td>Injection application</td>
</tr>
<tr>
<td></td>
<td>15°C</td>
<td>20°C</td>
</tr>
<tr>
<td>9</td>
<td>10.7</td>
<td>11.0</td>
</tr>
<tr>
<td>3</td>
<td>8.8</td>
<td>11.0</td>
</tr>
<tr>
<td>6</td>
<td>7.6</td>
<td>8.2</td>
</tr>
<tr>
<td>12</td>
<td>7.2</td>
<td>8.0</td>
</tr>
<tr>
<td>24</td>
<td>7.4</td>
<td>8.7</td>
</tr>
<tr>
<td>48</td>
<td>0.1</td>
<td>5.2</td>
</tr>
<tr>
<td>72</td>
<td>6.0</td>
<td>9.8</td>
</tr>
<tr>
<td>96</td>
<td>5.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>
of aziridines no doubt concerns the chromatin, the possibility of secondary effects upon the highly organized locomotory apparatus of spermatozoa cannot be entirely eliminated, especially at higher doses. In the course of an investigation of the fine structure of Dparopsis spermatozoa, the opportunity was therefore taken to look for possible structural abnormalities in sperm from tepa-treated males.

Dparopsis sperm have a mean length of 450 μ and a diameter varying between 0.2 and 0.5 μ. The head possesses a short acrosome which projects from the anterior end. The chromatin stains intensely and associated with the nucleus is a tubular structure, also noted by Yasuzumi and Onra [7] in Bombyx mori. From its size and position in Dparopsis this may possibly be associated with the acrosome.
### TABLE X. TEPA DEGRADATION IN MALE *Diparopsis*

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Method of treatment</th>
<th>Method of extraction</th>
<th>Mean squares</th>
<th>Regression equation</th>
<th>50% breakdown time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Regression Variance</td>
<td>Within dose variance</td>
<td>F ratio</td>
</tr>
<tr>
<td>10</td>
<td>injection</td>
<td>homogenisation</td>
<td>0.0013</td>
<td>0.0079</td>
<td>0.18</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>0.0040</td>
<td>0.0221</td>
<td>2.44</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td>0.0024</td>
<td>0.0333</td>
<td>0.28</td>
</tr>
<tr>
<td>15</td>
<td>homogenisation</td>
<td></td>
<td>0.0019</td>
<td>0.0214</td>
<td>0.58</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>0.0016</td>
<td>0.0203</td>
<td>4.04</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td>0.0077</td>
<td>0.0800</td>
<td>0.36</td>
</tr>
<tr>
<td>20</td>
<td>surface wash of</td>
<td>whole insects</td>
<td>0.0060</td>
<td>0.0544</td>
<td>1.12</td>
</tr>
</tbody>
</table>

### Figure 4. TEPA degradation of *Diparopsis* compared with the zero effect line.
Surrounding the posterior head and the middle regions is a radial mantle which takes the form of a series of petaloid structures. These structures, termed 'appendices laciniae' by André [8,9], who first described them in sperm of Pieris and Macroglossum, arise from the cell membrane and are finely striated transversely. Phillips [10] has observed in several lepidopteran species that these radial appendices are greatly reduced or lost from sperm in the ejaculatory duct, with the exception of one appendage which is recognizably different in having a reticular structure. This reticular appendage is also found in Diparopsis. Easily the largest organelles of the spermatozoon are the paired elongate mitochondrion structures which have conspicuous, regular clefts in their outer zone (Fig. 5). These giant mitochondrion structures are derived during spermatogenesis from the aggregation of mitochondria into nebenkern, the material of which reorganizes itself and elongates along the developing flagellum (see the review of Phillips [10]).

In Diparopsis, the rod-like mitochondrial derivatives are of unequal size and run alongside the flagellum for much of its length. In the terminal region the mitochondrial rods are tapered off, leaving the bare flagellum (Fig. 6). The flagellum possesses the familiar organization of two central fibrils with nine outer doublets. An additional nine single fibrils which stain more intensely are closely associated with the outer ring of doublets.

When whole air-dried spermatogonia are observed in the scanning electron microscope, the delicate radial appendages cannot be seen. Probably they have collapsed in the process of drying. Longitudinal divisions between the two mitochondrial rods and the flagellum are evident. If the preparation is shadow cast, regular transverse corrugations are revealed which may represent a close spiral organization of the mitochondrial cortex or the collapsed radial appendages (Fig. 7). Such a spiral organization cannot be deduced from the thin sections by transmission electron microscopy.

No evidence of damage to the delicate ultrastructure of the spermatogonia was observed three days after the injection of males with 10 μg of tepe, a treatment considerably in excess of the minimum sterilizing dose. Observations on agglutinated sperm from the spermatheca have yet to be completed.

**FIG. 4.** Total degradation of tepe in male moths after topical and injection methods of application compared with the rate of chemosterilant absorption through the cuticle.
FIG. 6. Transverse section through spermatocytes of two adjacent follicular cysts, showing the two mitochondrial derivatives (m₁, m₂), net-like structures of the radial mantle (p), the reticular appendage (r) and the axial filament (f). × 30,000

FIG. 6. Transverse section of spermatocytes showing the flagellum in the posterior region where the mitochondrial derivatives terminate. × 30,000
It should be observed that the spermatozoa of Dinaropsis are all fully formed when the adult emerges from the pupal stage. Thus tepa applied to the young adult cannot affect sperm development, only mature sperm structures. For the same reason, in this species, sterilized males are unlikely to recover fertility later in adult life.
DISCUSSION

To be of practical value a chemosterilant should not only be effective at very low levels, but also exhibit a wide margin between sterilizing and lethal doses. In addition the sterile insects must be competitive with those of the natural population and remain sterile throughout adult life. Finally, the rate of breakdown of the applied chemosterilant should be rapid to minimize possible environmental contamination.

The experiments described in this paper were designed to test whether these criteria could be satisfied in work on Diparopsis, as a prelude to field evaluation. When efficacy was expressed as the ratio between sterilizing and lethal doses, it was shown that apholate and tepa were significantly better candidates than metepa, hempa and triphenyl tin acetate.

The repetitive mating tests, however, clearly indicated tepa to be the more effective chemosterilant against male moths. Against female moths, on the other hand, the low sterility index of tepa indicated that sterility could only be achieved at a dose that would reduce mating efficiency. The competitiveness of male Diparopsis treated topically suggests that the release of sterile mass-reared or mass-collected insects would be feasible, although the females should be separated and eliminated.

The rate of tepa degradation after injection into male Diparopsis was rapid, as was that noted by Cox et al., [11], after oral application to the fall army worm moth, Spodoptera frugiperda, when estimated radio metrically. A slower rate of tepa degradation after topical application to the codling moth, Carpocapsa pomonella, was observed by Maltien and McDonough [12] when the treated moths were maintained in outdoor cages.

In Diparopsis the rate of degradation of topically applied tepa was appreciably slower than that of injected tepa. Thus at 20°C, 50% of a dose of 10 μg was metabolized in 19.3 h when injected, but in 73.3 h when applied topically. It is evident from Fig. 4 that the longer persistence of topically applied tepa is almost entirely due to its relatively slow rate of penetration into Diparopsis. The steady recruitment of applied tepa to the tissues by absorption through the cuticle may conceivably be of value in a species in which spermatozoa continue to mature for some days in adult life, but this is not the case in Diparopsis. It may be concluded that if male moths are sterilized by topical treatments of tepa in acetone and released shortly afterwards (necessary because of their short life) then some residual active tepa will be present on the cuticle at the time of release. Possibly the ingestion of tepa by probing would be a less hazardous procedure, since there is some evidence that absorption from the gut is more rapid than through the cuticle. The limitations of inducing sterility in Diparopsis by probing have, however, already been described (Campion [14], Campion and Outram [15]) and such a method does not seem to be of practical value. Topical treatments are, therefore, necessary.

Two factors operate to reduce the brief hazard that residues of topically applied tepa may represent. The first is that the combined effects of absorption and metabolism exhibit a high temperature coefficient (approximately 2.6 for topical treatments, compared with a Q₁₀ of 1.3 for injected treatments). Typical meteorological data from the cotton growing areas of Central Africa where Diparopsis is prevalent...
were reported by Tunstall, Sweeney and Rose [13]. The mean temperature in Makanga, Malawi, during the 1968/7 cotton-growing season, for example, was 28.5°C; in Gatoona, Rhodesia, it was 22.5°C. It is clear that at high temperatures approaching 30°C, a more rapid breakdown of tepa would occur, following the release of moths sterilized by some form of topical application, although at lower temperatures a much greater persistence would be expected.

The second factor is that Diparopsis is restricted to the cotton plant and is not found near food crops. Thus the release of tepa-sterilized moths is unlikely to have harmful effects on man or other mammals. Nevertheless, it is clearly desirable to ensure that virtually no traces of residual tepa enter the environment. It would certainly be worth while investigating the possibilities of improved formulation of tepa, e.g., by using oil solvents which, by diffusing over the whole insect, may promote faster penetration of the sterilant (cf. Lewis [16]). By such means a lower effective dose, a more rapid loss of tepa by metabolism and a consequent reduction in environmental contamination may be achieved.

This possibility is being investigated.

In spite of the observed agglutination of spermatozoa at very high doses, there was no evidence of secondary damage to the delicate ultrastructure at doses of tepa in excess of the minimum sterilizing dose. Damage to sperm structure may occur at even higher dose levels of the chemosterilant, where insect mortality is high, but because of the greatly reduced mating at these levels is unlikely to be of significance.

ACKNOWLEDGEMENTS

The authors are grateful to the Agricultural Research Council of Malawi for the supply of Diparopsis pupae, to Dr. A.B. Borkovec of the U.S. Department of Agriculture for the chemosterilant and to Mr. R.H. Williams for skilled assistance.

One of the authors (D.G.) wishes to thank Professor T.H. Southwood for working facilities provided at Imperial College Field Station, Silwood Park, Ascot.

REFERENCES

ÉTUDE À L'AIDE D'UNE TECHNIQUE D'IRRADIATION AUX RAYONS GAMMA DE LA MIGRATION ET DE L'UTILISATION DES SPERMATOZOIDES CHEZ LA BRUQUE DU HARICOT, Acanthoscelides obtectus (SAY)

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Abstract — Résumé

STUDY, USING THE GAMMA IRRADIATION TECHNIQUE, OF THE MIGRATION AND UTILIZATION OF SPERMATOZOA IN THE BEAN WEevil, Acanthoscelides obtectus (SAY).

Males of Acanthoscelides obtectus were irradiated with gamma rays from a cobalt-60 source; the dose used was 9600 rad. These males copulated normally, being introgressed into the reproductive systems of non-irradiated females. Spermatozoa containing all the paragametes and spermatocysts which migrate actively towards the spermatheca. The females have a normal fertility, but their fertility is nil: the embryonic development of the eggs laid stops at a very early stage. The successive use of sterile males and normal males provides a means of studying the migration and utilization of spermatozoa in the same female after two copulations. The spermatozoa deposited during the first copulation, whether by a normal male or by an irradiated male, all migrate into the spermatheca, but they are only partially utilized to fertilize the eggs; in all cases the spermatocysts deposited during the first copulation have a predominant role. It is probable that the mixing of the spermatozoa introduced into and stored in the spermatheca after two successive copulations would be only partial, even though incubation is halted for several days due to the absence of the bean seeds. The use of sterile males in the control of Acanthoscelides obtectus will be effective only if the females encountered are virgin. This would seem to be fairly rare under natural conditions: when the density of insects is high, the females mate a few hours after birth. The sterile males will therefore have to be introduced into the contaminated seed store before the adults emerge, but the effectiveness of this method of control may be diminished.

ÉTUDE À L'AIDE D'UNE TECHNIQUE D'IRRADIATION AUX RAYONS GAMMA DE LA MIGRATION ET DE L'UTILISATION DES SPERMATOZOIDES CHEZ LA BRUQUE DU HARICOT, Acanthoscelides obtectus (SAY).

Les mâles d'Acanthoscelides obtectus sont soumis à une irradiation de rayons gamma émis par une source de cobalt-60; le dose utilisé est de 9600 rad. Ces mâles copulent normalement; ils sont introduits dans la bourse copulatrice des femelles non irradiées en spermatocystes contenant toutes les paragamétites émises par les paragamétites, et les spermatozoïdes qui migrent activement vers la spermatheque. Les femelles ont une fécondité normale, mais leur fertilité est nulle; le développement embryonnaire des œufs est arrêté à un stade très prénéon. Un double copulation des mâles stériles et des mâles normaux permet d'étudier la migration et l'utilisation des spermatozoïdes, chez une même femelle, après deux copulations. Les spermatozoïdes, émis lors du second copulation par un mâle normal ou un mâle irradié, migrent tous dans la spermatheque, mais ne sont que partiellement utilisés pour féconder les œufs; dans tous les cas, les spermatozoïdes émis lors de la première copulation ont un rôle prédominant. Il est probable que le mélange des spermatozoïdes introduits et stockés dans la spermatheque après deux copulations successives ne soit que partiel, même lorsqu'il s'agit des œufs entrés, par suite de l'absence de graines de haricots, pendant plusieurs jours. L'utilisation de mâles stériles dans la lutte contre Acanthoscelides obtectus ne sera efficace que si les femelles rencontrées sont vierges. Ceci semble assez rare dans les conditions naturelles; lorsque la densité d'insectes est élevée, les mâles sont introduits quelques heures après leur naissance. Les mâles stériles doivent donc être introduits dans le sol de graines contaminées avant la sortie des adultes, mais l'efficacité de cette méthode de lutte peut être diminuée.
INTRODUCTION

Chez Acanthoscelides obtectus, lorsque deux mâles copulent successivement avec une même femelle, deux spermatozoïdes peuvent être déposés dans la bourse copulatrice. Après chaque accouplement, il y a stimulation nette de l’ovogénèse [1], mais la migration, puis l’utilisation des spermatozoïdes provenant de la seconde copulation n’ont pas été étudiées jusqu’à présent.

Un certain nombre de recherches de ce type ont été entreprises chez les insectes en utilisant successivement des mâles rendus stériles par une irradiation aux rayons X ou aux rayons gamma, puis des mâles normaux.

Les spermatozoïdes émis lors de la première copulation jouent un rôle prédominant au moment de la fertilisation des ovoocytes chez Glossina austeni [2], et chez Helistus virscens [3]. En étudiant Cylas formicarius Walker [4] constate que le premier accouplement est seul efficace. Chez d’autres insectes, tels que Trichophisia ni, [5], Carposcopas pannonella [6], Epilachna varivestis [7], les spermatozoïdes émis lors de la seconde copulation jouent un rôle prédominant, ou deviennent seuls efficaces au moment de la fertilisation des ovoocytes. Lefevre et Joneson [8], en étudiant la drosophile, pensent que les spermatozoïdes émis lors de la seconde copulation provoquent le déplacement d’une quantité plus ou moins importante de spermatozoïdes déjà présents dans la spermatheque et jouent de ce fait un rôle prédominant au moment de la fertilisation des ovoocytes.


L’influence des spermatozoïdes émis lors de la seconde copulation varie donc suivant les insectes. Au cours des expériences, nous étudierons la fertilité des femelles d’Acanthoscelides obtectus, mise en présence de mâles normaux puis de mâles rendus stériles par une irradiation aux rayons gamma (ou inversement).

1. METHODES


Les mâles sont séparés en deux lots, placés dans des conditions identiques dès leur naissance.

Premier lot: Les mâles sont soumis, à l’âge de 3 jours, à une irradiation de rayons gamma émis par une bombe au cobalt-60. La dose utilisée est de 9000 rad; après ce traitement, comme l’ont constaté Peason [12], Cavalloro et Bonfantil [13], tous les mâles sont stériles.

Deuxième lot: Tous les mâles sont normaux.

Tous les insectes utilisés au début des expériences sont âgés de 4 jours.

L’étude histologique de la bourse copulatrice des femelles est faite à l’Azan.

2. EFFETS DE LA RADIATION SUR LA FERTILITE DES MALES

Lorsque les mâles normaux copulent, 10–20% d’entre eux subissent une irradiation par les rayons X, que les rayons gamma ou alpha, qui sont absorbées par la bourse copulatrice, sont activées dans le corps des mâles normaux irradiés. L’irradiation modifie donc la fécondité.

La fécondité chez les mâles irradiés est nulle, les femelles irradiées ou non sont stériles. Les mâles non irradiés et les femelles normales produisent des femelles normales.

Ce phénomène n’a pas été observé chez d’autres espèces de drosophiles. Les mâles et les femelles irradiés produisent des femelles normales, mais les mâles irradiés ne peuvent pas produire de femelles normales.

Les résultats de ces expériences sont résumés dans le tableau suivant:

| Fécondité moyenne | 5 |
| Fertilité moyenne | 5 |
| Production ovarienne moyenne | 5 |

TABLEAU I. EFFETS DE LA RADIATION SUR LA FERTILITE DES MALES
L'étude histologique des spermatophores prélevés après les diverses copulations est faite sur coupes après fixation au Bouin, puis coloration à l'Azan.

2. EFFETS DE L'IRRADIATION SUR L'ACTIVITÉ REPRODUCTRICE DES MALES

Lorsque les mâles irradiés mis en présence de femelles vierges normales copulent, un spermatophore contenant les diverses sécrétions mâles élaborées par les paragones et les spermatocônes est déposé dans la bouche copulatrice de ces femelles. Les spermatocônes irradiés sont activés dans le spermatophore, à la fin de la copulation, puis migrent dans la spermathèque. La migration des spermatocônes est achevée, deux heures après la copulation, aussi bien chez les femelles ayant copulé avec un mâle normal, que chez les femelles ayant copulé avec un mâle irradié. L'irradiation des spermatocônes par les rayons gamma ne modifie donc pas leur activité.

La fécondité et la production ovarielle des femelles, isolées après une seule copulation avec un mâle irradié et placées en présence continue de grains de haricot (stimulus nécessaire à la ponte) sont identiques à celles des femelles témoins ayant copulé avec un mâle non traité. Les différences observées (tableau 1) ne sont pas significatives. L'introduction du spermatophore émis par le mâle irradié a donc normalement stimulé l'ovogenèse comme cela a été mis en évidence chez Acanthoscelides obtectus [1]. La fécondité, cependant, est nulle; le développement embryonnaire des œufs pondus s'arrête à un stade très précoce; il y a donc eu fertilisation des ovocytes par les spermatocônes irradiés. Grosch [14] pense que l'irradiation provoque des aberrations chromosomiques, au niveau des spermatocônes, et l'œuf fertilisé par ceux-ci ne peut se développer.

Ce phénomène nous permet d'étudier la migration puis l'utilisation des spermatocônes provenant de deux coupulations successives chez des femelles mises en présence d'un mâle normal et d'un mâle irradié. Les œufs ayant un développement embryonnaire complet ne peuvent être fertilisés, dans ces conditions, que des spermatocônes non irradiés.

**TABLEAUX I. EFFETS DE L'IRRADIATION SUR LE POUVOIR FÉCONDANT DES MALES**

<table>
<thead>
<tr>
<th>Femelles témoins (n = 70)</th>
<th>Femelles mises avec mâles irradiés (n = 50)</th>
<th>Valeur de t</th>
<th>Signification du test t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fécondité moyenne</td>
<td>57,3</td>
<td>57,6</td>
<td>0,14</td>
</tr>
<tr>
<td>Fertilité moyenne</td>
<td>51,7</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Production ovarienne moyenne</td>
<td>59,2</td>
<td>59,4</td>
<td>0,12</td>
</tr>
</tbody>
</table>
3. UTILISATION DE MALES IRRADIÉS ET DE MALES NORMAUX AU COURS D'EXPÉRIENCES DE COPULATIONS SUCCESSIVES AVEC DES FÉMELLES NON IRRADIÉES

3.1. Description des expériences réalisées

Les fémelles de la lignée II sont mises en présence d'un premier mâle au début de l'expérience, puis d'un second mâle quatre jours plus tard. Après chaque copulation le mâle est retiré et les fémelles sont isolées dans des boîtes d'élevage. Durant les six premiers jours les fémelles privées de graines de haricot n'émettent aucun œuf. Le sixième jour les fémelles sont mises en présence de graines de haricot et commencent à pondre.

Comme il n'y a eu aucun œuf émis jusqu'au sixième jour, on peut supposer, que l'œuf de la période de ponte, la spermatheque contient des spermatozoïdes, émis lors des deux copulations en quantités équivalentes. À ce moment, si le mélange était total, à l'intérieur de la spermatheque, tous les spermatozoïdes auraient autant de chances de fertiliser les ovocytes.

Les expériences sont réalisées sur trois lots d'insectes, de la même génération, placés dans des conditions rigoureusement identiques, pour limiter au maximum la variabilité (fig. 1).

Expérience 1 (témoin): Les fémelles sont mises en présence de deux mâles normaux.

Expérience 2: Les fémelles sont mises en présence d'un mâle normal puis d'un mâle irradié.

Expérience 3: Les fémelles sont mises en présence d'un mâle irradié puis d'un mâle normal.

<table>
<thead>
<tr>
<th>JOURS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>EXP1</td>
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<td>EXP2</td>
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<tr>
<td>EXP3</td>
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</tbody>
</table>

s≌: mâle normal
s△: mâle irradié

FIG. 1. Schéma des trois expériences.

3.2. Résultats obtenus

La fécondité élevée des mâles normaux est constatée. En étudiant quotidiennement les œufs, les dix premiers jours ont un taux de fécondité de 100%. Si le mâle est irradié, il ne se produit aucun œuf.

Utilisation des résultats

Au cours des expériences, on constate que le taux de développement de l'œuf est également différent. Les œufs des mâles normaux sont plus développés que ceux des mâles irradiés.

3.3. Résultats obtenus

En étudiant le taux de fécondité, on constate une différence significative. Le taux de fécondité des fémelles exposées à des mâles normaux est nettement plus élevé que celui des fémelles exposées à des mâles irradiés.
3.2. Résultats obtenus au cours de l'expérience témoin

La fécondité élevée, le septième jour, juste après l'introduction des stimulus de ponte, décroît progressivement au cours de l'expérience.

En étudiant quotidiennement l'ensemble des femelles pondéuses, on constate que le rapport r entre la fécondité et la fécondité varie peu durant les dix premiers jours de l'expérience; 10 à 15% des œufs émis ne sont pas fertilisés.

Durant les derniers jours de l'expérience, le rapport r diminue, le taux d'œufs non fertilisés augmente, mais cette variation est peu significative étant donné la fécondité individuelle de chaque femelle, très réduite à ce moment (tableau III).

Utilisation des résultats de l'expérience témoin dans le calcul de la fécondité théorique

Au cours de l'expérience utilisant successivement un mâle irradié et un mâle normal nous ne pouvons pas comparer les fécondités et les fertilités des femelles, car 10 à 15% des œufs n'ont pas été fertilisés et il est difficile de les distinguer des œufs ayant eu un développement embryonnaire avorté à cause des spermatozoïdes irradiés. Cependant, les femelles témoins peuvent nous servir de points de comparaison; nous pouvons supposer que la valeur quotidienne du rapport r serait susceptible de varier dans des proportions identiques si tous les mâles mis en présence des femelles lors des expériences 2 et 3 étaient normaux.

Dans ces conditions on peut déterminer quotidiennement, compte tenu de la fécondité de l'ensemble des femelles, la fécondité théorique. Le valeur de la fécondité théorique serait celle obtenue si tous les mâles mis en présence des femelles, lors des expériences 2 et 3, n'avaient subi aucune irradiation.

Cette fécondité théorique (f_th) pour le jour n sera:

\[ f_{th} = \frac{F_n}{r_n} \times r_n \]

\[ F_n = \text{fécondité des femelles en expérience le jour } n \]

\[ r_n = \text{rapport fécondité trouvé chez les femelles témoins le jour } n \]

La fécondité théorique nous servira de valeur de référence; la différence obtenue, le jour n, entre la fécondité théorique et la fécondité expérimentale, ne pourra être due qu'à l'influence des spermatozoïdes irradiés.

3.3. Résultats obtenus lors de l'expérience 2

En étudiant le tableau III, nous constatons que la fécondité expérimentale est nettement différente de la fécondité théorique; la valeur du X² (282) étant très supérieure à la valeur limite (18,46 pour 7 D.L.) pour un coefficient de sécurité de 99%.

Les spermatozoïdes irradiés issus de la seconde copulation migrent donc dans la spermatheque et sont utilisés pour la fertilisation des ovocytes.
TABLEAU II. RESULTATS DE L'EXPERIENCE 1. FEMELLES AVEC DEUX MALES NORMAUX

<table>
<thead>
<tr>
<th>Jours</th>
<th>J.7</th>
<th>J.8</th>
<th>J.9</th>
<th>J.10</th>
<th>J.11</th>
<th>J.12</th>
<th>J.13</th>
<th>J.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nombre de femelles pondues</td>
<td>27</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>35</td>
<td>35</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>fécondité</td>
<td>1163</td>
<td>452</td>
<td>303</td>
<td>317</td>
<td>291</td>
<td>698</td>
<td>190</td>
<td>152</td>
</tr>
<tr>
<td>Fertilité</td>
<td>1021</td>
<td>302</td>
<td>308</td>
<td>241</td>
<td>237</td>
<td>176</td>
<td>137</td>
<td>35</td>
</tr>
<tr>
<td>Rapport 1</td>
<td>0.87</td>
<td>0.84</td>
<td>0.78</td>
<td>0.76</td>
<td>0.91</td>
<td>0.67</td>
<td>0.68</td>
<td>0.54</td>
</tr>
</tbody>
</table>

TABLEAU III. RESULTATS DE L'EXPERIENCE 2. FEMELLES AVEC MALES NORMAUX, PUIS IRRADIES

<table>
<thead>
<tr>
<th>Jours</th>
<th>J.7</th>
<th>J.8</th>
<th>J.9</th>
<th>J.10</th>
<th>J.11</th>
<th>J.12</th>
<th>J.13</th>
<th>J.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nombre de femelles pondues</td>
<td>46</td>
<td>42</td>
<td>44</td>
<td>44</td>
<td>41</td>
<td>41</td>
<td>49</td>
<td>29</td>
</tr>
<tr>
<td>Fécondité</td>
<td>1417</td>
<td>575</td>
<td>566</td>
<td>428</td>
<td>322</td>
<td>293</td>
<td>290</td>
<td>156</td>
</tr>
<tr>
<td>Fertilité expérimentale</td>
<td>949</td>
<td>365</td>
<td>360</td>
<td>242</td>
<td>173</td>
<td>129</td>
<td>108</td>
<td>54</td>
</tr>
<tr>
<td>Fertilité théorique</td>
<td>1217</td>
<td>485</td>
<td>446</td>
<td>336</td>
<td>286</td>
<td>192</td>
<td>195</td>
<td>83</td>
</tr>
<tr>
<td>Rapport 12</td>
<td>0.64</td>
<td>0.73</td>
<td>0.71</td>
<td>0.74</td>
<td>0.86</td>
<td>0.89</td>
<td>0.67</td>
<td>0.65</td>
</tr>
</tbody>
</table>
FIG. 2. Évolution quotidienne de la fécondité théorique moyenne (C) et de la fécondité moyenne des femelles au cours de l'expérience (2). (Moyenne expérimentale ± 1 x t pour un coefficient de sécurité de 95%, 1 = 1,96 dans les conditions de l'expérience.)
### Tableau IV. Résultats de l'expérience 3. Femelles avec mâles irradiés, puis normaux

<table>
<thead>
<tr>
<th>Jours</th>
<th>1.7</th>
<th>1.8</th>
<th>1.9</th>
<th>1.10</th>
<th>1.11</th>
<th>1.12</th>
<th>1.13</th>
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<tr>
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<td>39</td>
<td>39</td>
<td>39</td>
<td>37</td>
<td>36</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Fécondité</td>
<td>1305</td>
<td>559</td>
<td>381</td>
<td>349</td>
<td>288</td>
<td>220</td>
<td>124</td>
<td>37</td>
</tr>
<tr>
<td>Fertilité expérimentale</td>
<td>446</td>
<td>158</td>
<td>111</td>
<td>82</td>
<td>76</td>
<td>50</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>Fertilité théorique</td>
<td>1233</td>
<td>472</td>
<td>308</td>
<td>241</td>
<td>218</td>
<td>149</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>Valeur de ( b )</td>
<td>0.37</td>
<td>0.33</td>
<td>0.26</td>
<td>0.24</td>
<td>0.34</td>
<td>0.23</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>Fert. exp.</td>
<td>Fert. th.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 3. - Étude quotidienne de la fertilité théorique moyenne (A) et de la fertilité moyenne des femelles au cours de l'expérience (B). (Moyenne expérimentale maximum actuelle pour un coefficient de sécurité de 99%; t = 1,66 dans les conditions de l'expérience.)
L'étude du rapport 10 entre la fertilité expérimentale et la fertilité théorique, relativement constant pendant toute la durée de l'expérience, montre que les spermatozoïdes non irradiés, issus de la première copulation, jouent un rôle prédominant dans la fertilisation des ovocytes. L'étude quotidienne de la fertilité théorique moyenne et de la fertilité moyenne des femelles au cours de l'expérience (fig. 2) confirme ce résultat ; 75% des œufs pondus sont fertilisés par les spermatozoïdes émis par le mâle normal.

3.4. Résultats obtenus lors de l'expérience 3

Nous constatons également que la fertilité expérimentale est très différente de la fertilité théorique, la valeur du X² (1106) étant très supérieure à la valeur limite (38,48 pour 7 D.L.) pour un coefficient de sécurité de 99%. Les spermatozoïdes normaux, émis lors de la seconde copulation pénètrent donc dans la spermathèque, mais ne jouent pas un rôle prédominant dans la fertilisation des ovocytes puisque le rapport 10 reste voisin de 0,35 durant toute l'expérience (tableau IV).

35% des œufs pondus ont donc été fertilisés par les spermatozoïdes émis par le mâle normal durant la seconde copulation ; la figure 3 montre bien la différence existant au cours de l'expérience entre la fertilité des femelles et la fertilité théorique.

3.5. Interprétation des résultats

Ces expériences montrent qu'une irradiation de 8000 rad ne modifie ni l'activité des spermatozoïdes irradiés, ni leur aptitude à fertiliser les ovocytes chez Acanthoscelides obesus. Dans tous les cas, les spermatozoïdes émis lors de la première copulation, qu'ils soient normaux ou irradiés, jouent un rôle prédominant dans la fertilisation des ovocytes.

L'étude de l'évolution journalière du rapport 10 et du rapport 1-10 (correspondant aux œufs fertilisés par les spermatozoïdes irradiés), montre que les deux courbes obtenues se superposent (fig. 4).

On doit admettre dans ces conditions, que 65 à 75% des œufs pondus par les femelles ont été fertilisés par les spermatozoïdes, déposés dans la spermathèque lors de la première copulation.


1) Etant donné l'état de répétition de la spermathèque, après le premier accouplement, on peut supposer que la migration des spermatozoïdes émis lors de la seconde copulation ne soit que partielle. La plus grande partie des spermatozoïdes, émis dans ces conditions, demeurait à l'intérieur du second spermatophore.

La spermathèque pourrait donc contenir un plus grand nombre de spermatozoïdes provenant du premier accouplement et ceux-ci auraient de plus grandes chances de fertiliser les ovocytes. En étudiant sur coupes histologiques des spermatophores prélevés vingt, quarante puis soixante minutes après la seconde copulation, on constate que la migration des spermatozoïdes est très retardée dans le deuxième spermatophore de la femelle ni dans l'accouplement.

Cette hypothèse n'est pas confirmée par les observations faites et la spermathèque contient une quantité importante de spermatozoïdes émis lors de la deuxième copulation.

2) On peut supposer que des spermatozoïdes ne soient pas seulement fertilisés par les spermatozoïdes de la spermathèque, mais que la spermathèque contient de nouveaux spermatozoïdes émis lors de la deuxième copulation.

Chez la drosophile, circulation continue de la spermatérophore au second spermatophore et réaccouplement pro proportion des spermatozoïdes émis dans la spermathèque au cours de l'accouplement.

Gugler, Kaplan et Pian, que des spermatozoïdes émis lors de la deuxième copulation, que la spermathèque, que les spermatozoïdes du spermatophore émis lors de l'accouplement.
l'ovaire et la fécondité de l'expérience, la première copulation des ovocytes.

Une et de la fécondité confirme ce résultat; le
spermatozoïdes émis par le

(lentement est très
évolutif) étant très
rapide; un coefficient de
lors de la seconde
n'est pas un
que le rapport 1,3
IV).

Les spermatozoïdes
la figure 3 montre
croître la fécondité des

spermatozoïdes est totale. On ne trouve pratiquement plus de spermatozoïdes dans le deuxième spermatophore déposé à l'intérieur de la houle copulatrice de la femelle ni dans les voies génitales soixante minutes après l'accouplement.

Cette hypothèse ne peut donc être admise: la spermatheque, au début de la période de ponte, doit sans doute contenir des quantités probablement équivalentes de spermatozoïdes émis par les deux mâles successifs.

2) On peut supposer que le mélange des spermatozoïdes provenant des deux copulations ne soit que partiel, les spermatozoïdes issus de la première copulation restant localisés dans la région du ductus receptaculi. Étant donné le fait de répulsion de la spermatheque, les spermatozoïdes en rétention sont en effet peu mobiles, et on peut concevoir dans ces conditions que le mélange se fasse difficilement. Flint et Kressin arrivent à la même conclusion chez Heliothis virescens [3], la première copulation joue également un rôle prédominant et le mélange des spermatozoïdes à l'intérieur de la spermatheque ne serait que partiel.

Chez la drosophile, Leveque et Jousson [8] pensent qu'il existerait une circulation continue des spermatozoïdes du réceptacle vers l'oviducte, et le réaccouplement provoquerait un déplacement en plus ou moins grande proportion des spermatozoïdes déjà présents dans la spermatheque.

Gugler, Kaplan et Kidd [15] ont d'ailleurs constaté, par autoradiographie, que des spermatozoïdes marqués avec de la désoxyguanosine détruite, émis lors de la seconde copulation, sont beaucoup plus abondants dans la spermatheque, que les spermatozoïdes non marqués provenant du premier accouplement. Ils ont donc plus de chances de fertiliser les ovocytes.

L'hypothèse d'un déplacement des spermatozoïdes, après la seconde copulation, peut être envisagée chez Acanthoscelides obtectus. L'arrivée de nouveaux spermatozoïdes dans la spermatheque provoquerait un déplacement du sperme déjà présent, puis à la fin de la migration, un rassemblement des spermatozoïdes émis par le premier mâle, dans la région du
ductus receptaculi. La densité des spermatozoïdes restant élevée, à
l’intérieur de la spermatheque, et leur mobilité réduite, on peut très bien
concevoir que le mélange des spermatozoïdes reste partiel pendant toute
la durée de l’expérience.

Une étude autoradiographique, à l’aide de spermatozoïdes marqués
par un isotope radioactif, devra être entreprise pour vérifier cette
hypothèse.

4. UTILISATION DES MALES IRRADIES DANS LA LUTTE BIOLOGIQUE
CONTRE ACANTHOSCELIDES OBCETUS

L’irradiation des mâles par les rayons gamma, à la dose de 9000 rad, ne
semble pas modifier leur comportement sexuel.

Des femelles vierges de la lignée II sont mises simultanément en pré-
sence d’un mâle vierge irradié, et d’un mâle vierge normal. Ces mâles
âgés de 4 jours, proviennent de la même génération de bruches sélectionnées.
Les femelles sont laissées en présence des mâles durant six heures, puis
sont isolées dans des boîtes d’élevage contenant des graisses de haricot.
Pendant six heures, 96% des femelles ne s’accouplent qu’avec un seul
mâle; leur bourse copulatrice ne contient d’ailleurs qu’un seul spermatophage.

Résultats de l’expérience: 182 femelles sont étudiées:
- 86 pendent des œufs fertiles et se sont donc accouplées avec un mâle normal
- 86 pendent des œufs dont le développement embryonnaire aorté
et ont donc copulé avec un mâle irradié.

Si l’accouplement se faisait au hasard chaque mâle aurait autant de
chances de copuler avec la femelle. 91 mâles normaux et 91 mâles irradiés
devraient donc copuler avec les 182 femelles.

Comparons les valeurs théoriques et expérimentales; le $\chi^2 (0,54)$ est
nettement inférieur à la valeur limite (6,64 pour 1 D.L.) pour un coefficient
de sécurité de 99%.

L’accouplement se fait donc au hasard et le mâle irradié a autant de
chances de rencontrer la femelle que le mâle normal, dans les conditions
de l’expérience. Les mâles stériles introduits dans une population de
bruches auront donc le même comportement sexuel que les mâles normaux.
Cependant, ils ne seront vraisemblablement pas copulatifs dans la lutte biologique que
s'ils s'accouplement avec des femelles vierges, puisque la première copula-
tion joue un rôle prédominant. Or, dans les lieux de stockage où les grains
sont contaminés la densité d'insectes est très élevée et les femelles
s'accouplent généralement quelques heures après leur naissance.

Les mâles stériles devront donc être introduits dans le stock de grains
contaminé avant la naissance des adultes. Ils entreront en compétition avec
les jeunes mâles normaux, né en même temps que les femelles; l'effi-
cacité de la lutte à l’aide des mâles irradiés contre les populations de
bruches du haricot risque, dans ces conditions, d'être diminuée.

CONCLUSIONS

Les mâles d'ACANTHOSCELIDES OBCEETUS sont soumis à une irradiation de
rayons gamma émis par une bombe au cobalt-60; la dose utilisée est de
9000 rad. Ces mâles copulent normalement; ils émettent dans la bourse
copulatrice de femel
les sécrétions élaborées
migrant activement vers
normale, mais leur
des œufs émis s’arrêter.

L'emploi suces de l'étudier la migration
femelle, après deux
mes accouplements,
dans la spermatheque
les ovocytes; dans les
mère copulation ont
des spermatozoïdes.
Copulations successives
des œufs est arrêté
plusieurs jours.

L'utilisation de
obceetus, ne sera effec
ci comme assez r.
d'insectes est élevé
naissance. Les mâ
de grains contaminé
méthode de lutte peu

[3] HIGNARD, J., C. b
[5] NORTH, B.T., RAN
[7] HENNEBERT, T., S.
[8] LEFEVRE, C., JOSIOI
[9] ZIORENE, L.D., BO
[10] GUNA, L.K., Nats
[12] PESSON, F., Indust. a
[13] CAVALLERONI, R., BON
[14] GROSCH, D.B., BOI

L. E. Le CHANC sperm from irradiat
from normal males,
dominantly utilized.
Why then do you con
excessive numbers o
be required?
catégorie, on peut très bien contempler pendant toute la durée de l'expérience des spermatozoïdes marqués et vérifier cette hypothèse.

**LUTTE BIOLOGIQUE**

Une dose de 9000 rad, administrée simultanément en pré- et post-réplication, est éclaircie. Ces mâles sont sélectionnés sur des larves de haricot, qu'avec un seul de ces mâles, une seule copulation puis une deuxième copulation sont efficaces. Soit deux copulations successives, et ce même lorsque l'émission est faible, et cette méthode de lutte peut être diminuée.

**REFERENCES**


**DISCUSSION**

I. E. Le CHANCE: in your double-mating studies you showed that sperm from irradiated males was utilized by females as well as that from normal males, but that the sperm from the first mating was predominantly utilized, regardless of whether the male was treated or not. Why then do you conclude that the sterile-male technique would require excessive numbers of released males, i.e. more than would normally be required?
J. HUIGNARD: The young males are usually more competitive than the irradiated males introduced into the contaminated stored grain, which are of course older. The sterile males could no doubt mate with a certain number of females, but they risk being ousted by the new-born males. That is why I think that the sterile-male method must have a reduced effectiveness unless a very large number of irradiated males are introduced.

M.E. TZANAKAKIS: Was the duration of the first mating of the female the same as that of the second?

J. HUIGNARD: Yes, the second mating lasts as long as the first, about seven minutes.
THE RESPONSE OF THE FEMALE ARTHROPOD'S REPRODUCTIVE SYSTEM TO RADIATION AND CHEMICAL AGENTS

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North Carolina State University,
Raleigh, N. C., United States of America

Abstract
THE RESPONSE OF THE FEMALE ARTHROPOD'S REPRODUCTIVE SYSTEM TO RADIATION AND CHEMICAL AGENTS.

This paper proposes more utilization of altered female reproductive performance when devising new approaches to population collapse of pests. It discusses results obtained and methods of analyzing the mode of action of a variety of chemical agents in addition to X and gamma rays.

An extremely important feature which determines whether a population will survive or collapse in response to a deleterious influence is the maximum number of offspring per female equal to the number of functional eggs produced. Fecundity data can be compared with the Wallace diagram of expectations from induced dominant and recessive mutational contribution to extinction.

Females in which ovaries failed to develop, or in which the ovaries have been destroyed may serve as the optimum sipper for sperm from a population's males provided females remain attractive and receptive. Peak vulnerability to destructive agents usually occurs during pre-imaginal development, but in some species where internal transformation lags behind external appearance, the opportunity for complete destruction exists in so-called adults.

The alternative situation is one in which the ovaries are fully mature. Two main categories are met here: (1) with oocytes already differentiated to provide for 1/6 or more of the lifetime egg deposit, or (2) with a minor portion of the potential eggs differentiated to oocytes but the major portion as undifferentiated germinal or stem cells. Because of strains uniquely suited to such investigations, most of the truly analytical experiments revealing differences in the responses of cell types have been performed in Drosophila, but the analysis can be extended to other species. Daily egg production records over an appreciable period are required. Then average performance for the most appropriate period of time is plotted. Also plotted is cumulative egg production. Characteristic curves distinguish between agents which cause chromosomal damage (radiations, activating agents) and those which function with nuclear cell function (antimetabolites). A quite different response is obtained with agents which attack somatic tissues (toxic metals, chlorinated hydrocarbons).

In conjunction with cytogenetic studies, information on egg hatchability provides important corroborative evidence. The analysis should not stop with scoring the unhatched eggs. A determination of stage of embryonic death may provide conclusive evidence on mode of action. The failure to complete cleavage and set up a blastoderm can be identified and distinguished from all other types of moribund conditions or stages of death. This feature is characteristic of parental chemosterilants which damage chromosomes.

1. INTRODUCTION

Although a few scattered references indicate that the sterilized female can play a positive role in a control program [1,2], the female has been the neglected sex. Two factors have contributed to the situation. Muller's classic techniques for detecting mutation employed treated males, and credit for the U.S.D.A. success in controlling the screw-worm has been attributed to the males released. This paper proposes greater utilization of altered female reproductive performance when devising new approaches to the collapse of pest populations. Auspiciously the active competitiveness necessary for males to carry genetic defects into a population is no problem when females are used in species where her role in mating is a passive one. She needs to be merely attractive and receptive to the male.
The consequences of introducing female sterility genes into a natural population has already been discussed (although briefly) at an I.A.E.A. symposium [1]. An extreme type of female sterility will be described below. In addition, the kinds of responses to chemical agents and radiations will be identified, and methods of analyzing their modes of action will be explained.

2. SIGNIFICANCE OF FECUNDITY

In our desire for means of rapid eradication we tend to lose sight of the important biological feature which determines whether a population will survive or collapse in response to a deleterious influence. This is the maximum number of offspring per female, in turn limited by the number of functional eggs produced. This paper directs attention to this subject.

Although a number of factors are involved in evaluating the damage from a mutagenic and cytotoxic agent, Chamberlain [3] found a composite equation possible if he employed a formula in which the important terms represent the number of viable eggs per female in the control and the treated group; furthermore, when the amount of dominant lethality has been determined from dose vs. effect experiments, it is possible to construct survival-extinction curves which show the average number of eggs each female must produce if the population to which she belongs is to persist. Laboratory experiments with Drosophila populations verify the predictive success of this theoretical treatment [4]. For genes with specific deleterious effects only when homozygous, a similar estimate is possible. Again it devolves to the number of eggs a female must produce in order to maintain heterotic systems involving a given number of independent gene loci [5].

Many insect types fall within the Drosophila fecundity range of 300 to 500 eggs per female. On the other hand, Anopheles mosquitoes which survive long enough to lay 10 batches of eggs can produce 2500 eggs each [6]. This impressive figure provides a reproductive potential equivalent to that of the primitive arthropod, Artemia salina. Nevertheless, because so much of this potential is a reserve used to buffer drastic shifts in environmental influences, only a fractional reduction in fecundity creates a situation precarious for the survival of Artemia populations [7].

3. COMPLETE ABSENCE OF OVARIIES

The ultimate state of infecundity is attained by females lacking functional ovaries. Females in which ovaries failed to develop, or in which ovaries have been destroyed could serve as the optimum type of siphon for sperm from males of an unwanted species.

The parasitic wasp Bracon hebetor is useful in biological control of Lepidopteran pests. As such, fecundity is desired. Our investigations are presented to exemplify extreme cases usually considered undesirable. Typically these are identified by a failure to oviposit despite avid feeding on the host caterpillar. Because of the parthenogenetic production of normal males, mating is not obligatory for oviposition. Upon dissection of this type of female, no ovarioles are evident but all other components of the reproductive system are present. These comprise the spermatheca and spermathecal glands, the lubricating gland, the poison glands and reservoir, the sting and all associated tubes and ducts. The condition can be the final stage of ovariole deterioration following exposure to a potent oksylating agent, or it may be caused by true gene mutation. One such case appeared several years ago in an imbed laboratory line. Easily maintained in the heterozygous condition, it differs morphologically from induced examples by the absence of the groups of tracheae ordinary formed ovarioles, ov end of the oviduct; evidence that such ty form of an oviduct may be responsible for such occurrence.

4. DESTRUCTION OF OVARIAN TISSUES

The intact tubes fill characterize the ovum within a week after is result from irradiation obtained by X-rays. Many of the eggs were short, horn-like.

In general, before the imaginal legs behind external the first few days of requirements are not producing females with material is destroyed secondary to the main stock. Many of the agents, have accessory of growth of the ovary.

5. A SOLUTION OF THE PROBLEM

Insects which into two main categories differentiated to pre (5) those with only oocytes. The former because differentiates surprisingly radiosensitive possibility of partis irradiated cytoplasm, the cell is fully de was of radiation. While it can do so in comb demonstrated in the (6): Figure 1 into the pre (7) 10 female brachyom of 3-dialo-5-mono-1-m dissolving 10 mg of I solution. As shown, concentrations until egg production climb.

To determine result from cell dest higher concentration day. Within 24 hours accumulating region deterioration of the opositional masses in ti within the sheaths.
of tracheae ordinarily serving the ovarioles. Following the destruction of formed ovarioles, obvious bundles of tracheoles are found close to the distal end of the oviduct. The presence of sperm in the spermathecae have provided evidence that such types attract mates and allow mating.

4. DESTRUCTION OF OVARIOLE CONTENTS

Tissues of the ovariole sheath are insensitive to ionizing radiation. The intact tubes filled with degenerating nurse cells and cytoplasmic debris characterize the ovarioles in Bracon [8], Drosophila [9], and Callitroga [10] within a week after 5000 R or more. Even more comprehensive destruction can result from irradiating pre-imaginal insects. A most impressive example was obtained by X-raying Bracon pupae. Instead of ovarioles, the adult structures were short, horn-like sacs filled with acellular debris [11].

In general, peak vulnerability of the ovariole contents occurs before the imaginal stage. But in some species where internal transformation lags behind external appearance, immature ovarioles are still available during the first few days of adulthood (flies) or until special nutritional requirements are met for maturation (mosquitoes). Thus the opportunity for producing females without ovarioles exists in so-called adults. Whether formed material is destroyed or whether the development of the ovary is halted is secondary to the main purpose of producing females which lack ovarioles. Amethopterin, nitrogen mustard, and colchicine, three quite different types of agents, have accomplished this in houseflies [12] by interfering with growth of the ovary, each agent in its own fashion.

5. A SOLUTION OF THE PROBLEM OF DIFFERENTIATED OOCYTES

Insects which develop fully matured ovarioles before adulthood fall into two main categories: (1) those in which sufficient oocytes are already differentiated to provide a third or more of the lifetime egg deposit, or (2) those with only a minor portion of the potential eggs differentiated to oocytes. The former appears to pose more of a problem in insect control because differentiated oocytes in certain stages of mitotic prophase are surprisingly radioresistant. A further complication is added by the possibility of parthenogenetic development of the sperma nucleus in heavily irradiated cytoplasm [13]. Once the follicle-enclosed, trophocyte-massaged oocyte is fully developed it tends to produce an ovum despite massive doses of radiation. While radiation alone cannot halt egg production completely it can do so in combination with a carefully selected antimitobolite as demonstrated in the following experiment.

Figure 1 presents the average egg production of 6 samples of 10 female braconids each. Five of the samples ingested a single meal of 6-diace-5-oxo-1-norleucine (DON) from a dilution series prepared by dissolving 10 mg of DON in 25,50,100,200, and 400 ml of saturated sugar solution. As shown, no eggs were deposited by females fed the two higher concentrations until the fourth and fifth days respectively. Subsequently egg production climbed quickly to control levels.

To determine whether this pattern of oviposition does indeed result from cell destruction, additional samples of 30 wasps were fed the higher concentrations. Of these samples five females were dissected each day. Within 24 hours only diffuse granular debris remained in the egg accumulating region of the ovarioles. Subsequently within 2 to 3 days degeneration of the entire oocyte sequence became evident. Except for the oogonial masses in the blind ends of the tubes, no formed elements persisted within the sheaths.
Repopulation of the ovarioles dominated the histological aspect on the fourth and fifth days. A complete polyploidy sequence filled the ovarioles before oviposition began again. The pattern of response to DOM is in direct contrast to that observed after delivery of X or gamma rays. After irradiation intra-ovarial debris is slower to appear and it is the degeneration of the distal end of the ovariole which are destroyed [8]. Therefore if completely inseminated females are desired, the two agents complement each other exactly. Figure 2 shows what happens when a radiation dose which halts egg production after five days is combined with a DON dose which eliminates egg production for the first five days. A group of females was produced which never laid any eggs. The fecundity of their sisters is shown by the control data plotted as well as by the impressive oviposition of the DOM group after the eighth day.

6. REVEALING DIFFERENCES IN CELL TYPE SENSITIVITY

Now we will consider situations in which only part of the ovariole contents are destroyed. A decrease in the total number of eggs deposited by a group of females indicates nothing more than a deleteriousness of the agent used. It may even cause destruction of the germ plasm of the gonad tube. The entire period to include eggs derived well as the most high of the females of the functional ovaries, an

LaChance's is hominivorax [10,14,15] contains more than 100 egg masses. Perhaps it is a sparse literature, correlating them with others in a determined study on the screw worm, a similar species; in which case 7 e ovariole groups were regrouped. Particular experiments by Abrahams of shorter periods a do not receiving the acute dose have little effect on oviposition rate in [17].

In contrast to egg production from an uniquely suitable location in the wasp's Brie fly these females are (1) moderate radiation exposure ability of transition
agent used. It may even reflect damage to somatic organ systems rather than destruction of the germ line. Experiments to reveal differences in the responses of the gonad's cell types require egg-production records per unit time. The entire period of time covered by such records must be long enough to include eggs derived from the most primitive type of precursor cell as well as the most highly differentiated kind of oocyte. In addition, the females of the groups compared must possess an equivalent number of functional ovaries, and only fecund females are considered.

LaChance's laboratory applied this approach to Cochliomyia hominivorax [10, 14, 15], accomplishing the task for a pest in which each ovary contains more than 100 ovarioles, each of which can produce an egg for each egg mass. Perhaps it is the labor more than any other factor which explains a sparse literature. Be that as it may, by sequencing the deposits and correlating them with a cytological study of ovariole contents, LaChance succeeded in determining the comparative radiosensitivity of cell types.

At the same time he established the vulnerability of trophocyte endomitosis in the screwworm, a substantiation of earlier reports on Bracon [8]. Furthermore, certain Drosophila data provide additional corroboration when regrouped. Particularly interesting are the egg deposits of the 3000 R experiment by Abrahamson and Herkowitz [16]. When pooled by days instead of shorter periods a decline during the first week becomes obvious for females receiving the acute dose. In contrast fractional delivery of the same dose has little effect. Unfortunately no one has made the 30 to 40 day study of oviposition rate in treated Drosophila as recommended nearly 40 years ago [17].

In contrast to many other insects considered, Habrobracon's modest egg production from only two synchronized ovarioles per ovary has proved uniquely suitable for analytical experiments. Five distinct types of modification in the wasp's oviposition pattern were identified previously [18]. Briefly these are (1) the family of two-looped curves obtained with low and moderate radiation exposures. The interposed valleys attest to the vulnerability of transitional cells in mitosis [19]. Recently similar curves have
been obtained with a potent alkylating chemosterilant, apholate [20]. Higher doses of radiation destroy the oogonia. This kind of response, designated II, was shown on Figure 2. A third situation (III), a constant deficit in eggs every day seems to be characteristic of somatic debility. This has been produced by Fourth Period cation poisoning, classic organic enzyme inhibitors, and recently by heptachlor, a chlorinated hydrocarbon insecticide [21]. Completely different organ systems may be attacked by the different kinds of agents. The fourth kind of pattern (IV) is induced by antiestrogens which cause a valley to develop two to three days earlier than a radiation induced low point, i.e. a low by the third day [22]. Still another type of curve (V) has been obtained with agents which temporarily inhibit mitosis [23]. In this case there is a compensatory deposit of eggs which makes up for earlier deficits. The present report of DON destruction of oocytes adds a sixth kind of curve (VI) in which egg production starts at zero for the first five days and then rises to control levels. A lower total number of eggs deposited may be due to any one of at least four kinds of modifications in egg production. Figure 3 demonstrates how a 100 egg deficit might result.

Whereas a plot of the day-to-day average serves to reveal differences in the sensitivity of the cell types by demonstrable peaks and valleys, plotting the cumulative total enables a rate comparison, reflected in the slopes of the lines. Also this kind of plot opens the door to statistical analysis, via the methods of linear regression. For Bracco the approach has been helpful in analyzing the reproductive performance of females from Bisectalita experiments [24]. The typical radiation response shows an obvious inflection and lower rate of egg deposit from days 4 to 10; subsequently, the rate returns nearly to control levels. Figure 4 shows data plotted after groups of females were subjected to one of several agents. The response is quite different from that to irradiation. The daily deficit for wasps poisoned with Ni + or with arsenite is reflected in a lower rate of deposit, and hence divergence from the control line. Methotrexate, a folic acid antagonist, induces a very low rate of egg production until after the sixth day, although subsequently in the second week an appreciable slope is apparent.
100 egg deficits from
sted in the text. For the
or can serve as an example.

olate [20].

response,
III, a constant
cromatic deblility.
classic organic
t hydrocarbon
be attacked by
(IV) is induced
three days earlier
third day [22]. Still
which temporarily
very deposit of eggs
of DON destruction
production stays
levels. A
of at least four
estimates how a

FIG. 4. Cumulative egg production. Each line plots the average for a sample of 20 bracoids. "As" designates the results from a single meal of 0.05% sodium arsenite. "Ni" indicates results after an 0.05% NiSO₄ feeding. "Meth" identifies results after a 0.05% methionine feeding. All solutions were in sugar water presented at time zero.

TABLE 1. THE PERCENTAGE OF EGGS WHICH HATCHED AFTER DEPOSIT BY BRACON FEMALES FED CERTAIN CHEMICAL AGENTS OR IRRADIATED

<table>
<thead>
<tr>
<th>Cell Type From Which Derived</th>
<th>Oocytes</th>
<th>Transitional</th>
<th>Oogonia</th>
<th>Oogonia (Sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deposited on Days</td>
<td>1-5</td>
<td>6-10</td>
<td>11-15</td>
<td>16-20</td>
</tr>
<tr>
<td>Control</td>
<td>98.1</td>
<td>95.6</td>
<td>93.4</td>
<td>78.5</td>
</tr>
<tr>
<td>DON</td>
<td>-</td>
<td>91.5</td>
<td>85.9</td>
<td>78.7</td>
</tr>
<tr>
<td>NaAsO₄ 0.05%</td>
<td>91.2</td>
<td>88.6</td>
<td>88.3</td>
<td>78.5</td>
</tr>
<tr>
<td>NiSO₄ 0.05%</td>
<td>88.2</td>
<td>81.5</td>
<td>80.7</td>
<td>82.5</td>
</tr>
<tr>
<td>2500 R</td>
<td>75.3</td>
<td>67.0</td>
<td>80.1</td>
<td>58.9</td>
</tr>
<tr>
<td>Co-60</td>
<td>84.8</td>
<td>80.3</td>
<td>78.6</td>
<td>72.9</td>
</tr>
</tbody>
</table>
7. CORROBORATION FROM HATCHABILITY AND STAGE OF DEATH

Information on egg hatchability and the stage achieved before death of the embryo is important for establishing an agent's mode of action. Most significant as an indication of inducers of chromosome aberration and gross nuclear damage is poor hatchability in which a majority of embryos died in what Von Borstel's laboratory has called Stage 1 Death [25].

Not every chemical agent which interferes with egg production induces damage in genetic mechanisms. As evidence of this, Table 1 presents hatchability summarized by five day periods to correspond to the cell type in the ovaries at the time of treatment. This recognizes that eggs destined to be deposited on a particular day were in one of the precursory stages on the initial day of an experiment.

An additional category, Days 16-20, is distinguished on the basis of age of the mothers. B. devastor females typically enter their senile decline on the 15th day, as is reflected in the decreased hatchability of control groups of eggs.

Neither DCM nor NaAsO₂ lowered hatchability impressively. An initial low hatchability, such as that produced by Ni²⁺ assumes characteristic of toxic fourth period cations (unpublished data), evidently due to their incorporation into the oocytes. For contrast, data from a radiation experiment is shown. Egg hatchability is impressively lower throughout the reproductive period of the irradiated females.

A further difference in mode of action is revealed when a classification of the stages of death is considered. After 2000 R of Co-60 gamma rays a majority (66%) of the embryos were classifiable as Stage 1 Deaths. On the other hand, this type of embryonic death was not obtained as a response to the chemical agents of Table 1. The rare cases seen did not even amount to control proportions of 6% during the first five days. With maternal aging this decreased to 2% for the senile period. In other words, except in the radiation experiment, embryo deaths occurred late in development. Stage 1 Death results from an inability to accomplish cleavage. Instead of a cleared egg periphery representing blastoderm formation, Stage 1 Death is a non-homogeneous, cloudy white moribund state. As the most frequent kind of radiation-induced dominant lethality, something equivalent to the bracoid example presumably characterizes the syngiotic deaths inherent in the acellular "sterility" approach. Bracoid merely provides exceptionally suitable material for detecting deviations from the normal course of development.

The thin chorion is so transparent that a microscopist can see exactly what is happening inside an egg without recourse to microtechnique.

8. RESPONSE TO THE INHIBITION OF DNA SYNTHESIS

More subtle responses in cell type sensitivity than those yielding deficits of 100 eggs or more can be detected by the careful sequencing of egg production. An extremely important matter in our understanding of the operation of the polytrophic override is the time of DNA synthesis.

Beginning with tests of FUDR by Dr. Roger H. Smith (M.S. Thesis, North Carolina State University, 1962), experiments subjecting bracoid females to a variety of DNA inhibitors have been performed. Several herbicides including propamill seemed ineffective, as well as the antibiotic novobiocin. Perhaps some of these compounds need to be reconsidered from the perspective of recent experiments with hydroxyurea.

FIG. 5. Contrast between the 6 females after exposure to two 25" gateways with hydroxyurea. 0.1 ml of samples are plotted after the sample shown.

Hydroxyurea is mammalian tissue culture cytosid effectiveness and a wide variety of normal invertebrates and vertebrates cell-destruction expert group of cells in the o
achieved before death of the embryo. Most of the embryos died in a few days. The egg production data, Table 1, presents the average number of eggs produced by the cell type. The eggs destined for the recrudescent stages are shown on the basis of their ability to cleave and reform the cell. An analysis of the data reveals that the number of eggs per female is almost constant throughout the period of observation. When a class of 100,000 eggs was sacrificed at Stage 1, it was not observed that the eggs would cleave and form the cell. As the eggs were at Stage 1, the equivalent of a 100,000 eggs was sacrificed at Stage 1. The equivalent of an egg at Stage 1 was sacrificed at Stage 1. The equivalent of an egg at Stage 1 was sacrificed at Stage 1. The equivalent of an egg at Stage 1 was sacrificed at Stage 1. The equivalent of an egg at Stage 1 was sacrificed at Stage 1. The equivalent of an egg at Stage 1 was sacrificed at Stage 1.

Hydroxyurea is the currently preferred means of synchronizing mammalian tissue cultures because it kills cells in G1 phase (26). Its cytotoxic effectiveness has been demonstrated in vivo on neoplasms and on a wide variety of normal cell types including bacteria, protozoa, plant roots, invertebrate and vertebrate embryos, and mammalian bone marrow (27). How cell-destruction experiments with Hydroxyurea have identified a particular group of cells in the ovariole germarium which are sensitive to hydroxyurea.

The most effective type of experiment employed two radiation exposures separated by a four-hour period, during which an injection had assured presence of hydroxyurea in the female abdomen. Cu 137 provided the gamma rays. Figure 5 shows how similar oviposition by two injected groups is to that of an un.injected group until the 10th day after the radiated dose of radiation. Then hydroxyurea caused a sharp drop. On the 12th day or earlier eggs were deposited than by vials given the acute dose of 5000 R in two minutes. Hydroxyurea alone produces a slight decrease from the control level of egg production between 12 and 14 days after injection. The more pronounced effect in the traditional test for radiation recovery suggests that hydroxyurea either interferes with chromosomal rejoining mechanisms or promotes the formation of actual lesions at provisional sites of damage.
9. APPLICABILITY STATEMENT

The primary purpose here has been to contribute to our understanding of the insect’s reproductive physiology. Nevertheless, in addition to eliminating the necessity of rearing pupae before release, as has been the standard procedure in manipulating screwworm populations, alternative uses of treated females suggest themselves. The incorporation of infertile genotypes, as well as other cytogenetic features in complex stocks derived for insect control could enhance the effectiveness of the method, provided appearance of the defect is devised for subsequent generations. This is the delayed or "time-bomb" approach. Quite different in concept is the direct killing of the 'booby-trap' technique, wherein infertile females from a pesticide resistant strain are coated with an agent which kills susceptible males during mating attempts (2). The ingenuity of genetically trained entomologists may be expected to provide additional applications.

10. SUMMARY AND CONCLUSIONS

After discussing the significance of female fecundity in survival and extinction situations, extreme types of infertile genotypes were described, as well as methods for the destruction of the ovariole contents. More decrease in numbers of eggs deposited is completely inadequate for making a decision concerning the mode of action of a tested chemical agent. For a rational understanding of an agent's potency and limitations there is a dearth of information. Data is needed on sequenced egg deposits and the effective lethal phase in the embryos. Events even as subtle as the S period preceding the mitotic activity of the cytoplasm can be revealed. This area of biology is relatively unexplored both from the standpoint of investigation and application.

ACKNOWLEDGMENT

Support for studying the mode of action of chemical agents upon reproductive performance is provided by U.S.P.H.S. grant ES-00044, Division of Environmental Engineering and Food Protection.

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[25] VON BORSTEL, R. C.
[26] SINCLAIR, W. K., S
[27] THURMAN, W. G., (E

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L. E. LaCHANCE: of DON on mature oocy
D. S. GROSCH: of highly-differentiated o
observations on oari
an and one that m

R. PAL: Some of in connection with the

**DISCUSSION**

L. E. Lachance: Would you care to comment on the mode of action of DON on mature oocytes?

D. S. Grosch: Our first concern was to make sure that the large, highly-differentiated oocytes were indeed induced to degenerate. Our observations on osorption are given in the text of the paper. Since synthesis and the incorporation of stored materials is practically complete, it is difficult to see how an antimetabolite could have any direct influence on the structure. Indirect mechanisms, including hormonal controls, should be investigated. Furthermore, it should be noted that these were feeding experiments, for which partial starvation is a necessary preliminary, and one that interferes with any accumulation of stored metaphase eggs.

R. Fal: Some of the work carried out by Soviet research workers in connection with the development of resistance to insecticides has shown
that DDT affects oogenesis in house flies. Perhaps Mr. Grosch would like to comment on these findings.

D. S. GROSCH: The literature on this subject appears to fall into two categories. Firstly, there are observations on populations subjected to DDT for more than one generation, where the very powerful genetic influence of selection has had an opportunity to act. Secondly, there is information on the exposure of flies with immature ovaries. In this case DDT acts upon a developmental situation which depends upon the functioning of the somatic metabolism. Dr. Beard of the Connecticut Experiment Station (USA) has published some observations on this matter.

The decreased average egg production shown in the figures reflects poor performance by poisoned females. The somatic fitness of the mother is easily influenced by pesticide.

Abstract

CHEMOSTERILANT EFFECTS ON Stomoxys calcitrans (L.). Histological aspects of the fly have been investigated.

Larvae were more tolerant age, older individuals being less periods and these pupae were of the larval anatomy of the major developmental disc and following topical treatment with some of the factors influence reported in detail. The four rate of antennae growth: (2) Aberrant eyes and aristapedia mutant eyes. This last damage was confirma by tests, possibly may influence this process, but

INTRODUCTION

Population control insects has received a following the success of Anophelines and Stomoxys (Coquerell) the United States of America which have absorbed then of sterility in the adult the female. A survey devoted to their action

Many compounds have sterilizing, toxic prop domestica L. [1] and th

In the few investigated cellular damage can have been observed: gonal development induction of chromosome [4], and delayed break
CHEMOSTERILANT EFFECTS ON THE LARVAL POSTEMBRYONIC DEVELOPMENT OF THE STABLE FLY, Stomoxys calcitrans (L.)

J. S. BADWIN
Imperial College Field Station, Ashurst Lodge, Sunninghill, Berks, United Kingdom

Abstract

CHEMOSTERILANT EFFECTS ON THE LARVAL POSTEMBRYONIC DEVELOPMENT OF THE STABLE FLY, Stomoxys calcitrans (L.).

Histological aspects of the action of tops on the postembryonic developmental system of the stable fly have been investigated.

Larvae were more susceptible than adults to the toxic effects of tops although their sensitivity varied with age, older individuals being less affected. Treated larvae pupated later than controls and over longer periods and these pupae were often partially vacated or malformed.

The larval anatomy of Stomoxys calcitrans (L.) is described with special reference to the organisation of the major developmental discs. An overall reduction in the size of these primordia was observed following topical treatment of the larvae; this effect lessening with later application.

Some of the factors influencing normal growth and differentiation of the eye-antennal discs are reported in detail. The four main effects of chemosterilant damage to this disc are: (1) A reduction in the rate of antenna growth; (2) Abnormal differential growth of the disc; (3) 3 and 4alar treated larvae emerged as eyeless and anisotropic mutants; (4) Late larval treated larvae emerged as normal adults with defective eyes. This last damage was confined to a ventral band of abnormally arranged facets on the cornea. Successive larval treatments with tops caused this pattern of damage to extend anteriorly across the eye. Factors which may influence this process, including possible hormonal control, are discussed.

INTRODUCTION

Population control by the release of partially or completely sterile insects has received an increasing amount of attention during recent years following the successful eradication of the screw-worm, Cochliomyia hominivorax (Coquerel) from the West Indian island of Curacao and from the United States of America. The major characteristics of chemosterilants which have absorbed the interests of workers in this field are the induction of sterility in the adult male and the inhibition of ovarian development in the female. A survey of the literature shows that little attention has been devoted to their action on other stages of development.

Many compounds which are effective adult sterilants have revealed low sterilizing, toxic properties when applied to larvae of the house-fly, Musca domestica L. [1] and the screw-worm fly C. hominivorax (Coquerel) [2].

In the few investigations that have been reported, the following types of cellular damage caused by chemosterilant applied to the larval stage have been observed: delayed induction of sterility and interference with gonadal development in Culex pipiens quinquefasciatus Say by pharmac [3]; induction of chromosomal aberrations, including fragmentation and stickiness [4], and delayed breakdown of nerve fibres in the adult brain and degeneration...
of the mid gut epithelium in *Aedes aegypti* (L.) by aholate [5]; a lower rate of chemosterilant breakdown than that exhibited by adult *Culex tarsalis* Coquillett [8] and interference with postembryonic development of the cockroach *Carcopis cauponella* (L.) by tepa [7].

An advantage of studying the effects of chemosterilants on the larval stage is that many developmental systems can be observed simultaneously in the same insect. In Diptera a large proportion of the external features of the adult arise from the embryonic hypoderm as discrete groups of actively dividing cells and continue their development as imaginal discs throughout the remaining larval period.

In the present study several effects of tepa (tris (1-aziridinyl) phosphine oxide) on postembryonic development of the eye-antennal disc of the stable fly, *Stomoxys calcitrans* (L.), are described. For comparison, complete sterility of the adult fly has been achieved by topical application of 3.7 µg tepa [8].

**TECHNIQUES**

Eggs of *S. calcitrans* deposited over a one-hour period were transferred to dishes containing the culture medium (50 larvae per 50 g food) and maintained at 27°C, 70% r. h., Hopkins' method [9] of culture was followed.

Under these conditions, with variations in hatching time and variable rates of larval development, both the first and second instar lasted approximately 24 hours and the entire larval period covered 10-12 days.

Under experimental conditions 0.5 µl of tepa:acetone solution from a micro drop applicator were applied topically to individual larvae of the required age, which were then returned to the dishes after a holding period of 10 min. Owing to the small size of both first and second instars it was found easier to mix a measured amount of aqueous tepa solution, ranging from 0.1-100 ppm, into the culture medium than to apply it topically.

**TOXICITY**

Larval and pupal LD₅₀'s for tepa treatments applied during each instar of *S. calcitrans* are represented in Table I. The results show an increasing tolerance during the early stages of larval development from 8 µg (first instar), 9 µg (second instar), to 34.5 µg midway through the final larval stadium. The remaining period of larval growth up to the time of pupation shows a decline in tolerance to 17 µg.

Between 0.5 and 100 µg tepa, duration of the larval period was extended in direct proportion to the dose applied, as shown by Fig.1. Further evidence for a decline in tolerance before pupation was given by measurements of the amount of chemosterilant required to extend the larval period by 50%. Mid-third instar larvae required 7.1 µg tepa, while a similar delay in pupation of a later stage of this instar needed 1.15 µg.

In all these experiments there were considerable numbers of partially tanned or mini-shaped puparia especially at high doses. The frequency of occurrence of these distorted specimens also increased directly with the amount of chemosterilant used.

**TABLE I.**  LD₅₀ OF S. EXPRESSED IN µG, x

<table>
<thead>
<tr>
<th>Stage of application</th>
<th>x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instar I</td>
<td>8</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
</tr>
<tr>
<td>III day 1</td>
<td>34.5</td>
</tr>
<tr>
<td>day 3</td>
<td>17</td>
</tr>
<tr>
<td>day 5</td>
<td>17</td>
</tr>
<tr>
<td>day 7</td>
<td>17</td>
</tr>
</tbody>
</table>

**FIG. 1.** Mean Increase in the larval period in days
- O applied on day 3
- □ applied on day 7

**DEVELOPMENT OF T.**

Overall growth of development was slightly dissected discus under a microscope. Usually five at any one dose.
plate [5]; a lower rate of development of the coding by low dose treatment with TEPA to Culex tarsalis larvae simultaneously applied to the external features of several discs of the cephalic or frontal disc of the stable of these imaginal discs of 3.7 μg/kg. Period were transverse per 50 g food) of culture was long time and variable instar lasted covered 10 -12 days, one solution from a dual larvae of the after a holding period, and second instars at a dose that can to apply it topically.

and during each instar show an increasing body from 6 μg (first in the final larval stage of the time of pupation and period was extended Fig.1. Further given by measures end the larval period while a similar delay in numbers of partially grown. The frequency of and directly with the

<table>
<thead>
<tr>
<th>Stage of application</th>
<th>Larval mortality</th>
<th>Pupal mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instar I</td>
<td>2 - 5.85</td>
<td>1 - 2.8</td>
</tr>
<tr>
<td>II</td>
<td>3 - 9.1</td>
<td>1 - 3.0</td>
</tr>
<tr>
<td>III day 1</td>
<td>15</td>
<td>0.13</td>
</tr>
<tr>
<td>day 3</td>
<td>34.5</td>
<td>3.3</td>
</tr>
<tr>
<td>day 5</td>
<td>15.1</td>
<td>2.17</td>
</tr>
<tr>
<td>day 7</td>
<td>17.3</td>
<td>1.37</td>
</tr>
</tbody>
</table>

FIG. 1. Mean increase in the larval period of Stomoxys calcitrans (L.) after topical application of TEPA. ⊗ applied on day 5 ● applied on day 11

DEVELOPMENT OF THE CEPHALIC DISC

Overall growth of the cephalic or frontal disc during postembryonic development was studied by measuring the relative increase in area of dissected discs under a stereoscopic microscope with an ocular micrometer. Usually five specimens were measured from each of three replicates at any one dose.
FIG. 2. Increase in relative cross-sectional area of the cephalic disc during larval life, each point representing the mean of five measurements. Disc area was calculated from the product of the diameters of the two major axes.

- control
- 10 μg

On hatching, the rudiments of the cephalic disc in S. calcitrans are well established. Growth is slow at first, but during the second instar the disc begins to alter shape into a small proximal and broad distal section and these areas subsequently differentiate into the antennal and eye regions respectively. Development within the final larval instar is characterized by an accelerating growth phase and the initiation of ommatidial differentiation.

Topical application of 10 μg tepa at the beginning of the third instar resulted in a considerable suppression of growth as shown by Fig. 2. Up to the time of pupation in the control dishes, growth of the treated discs was slow but otherwise apparently normal. Later, especially after first and second instar treatments, imaginal discs developed enlarged antennal or optic regions which may have been capable of further differentiation. Above 10 μg tepa the frontal disc failed to differentiate correctly, its rigid structure collapsed and a darkened mass of cells appeared in its centre. Sections of this material revealed severe destruction and disorientation of the imaginal cells.

Although the disc comparison with the cephalic disc, the ch optic anlagen being a structural collapse or be a consequence of action coupled with the surrounding tissue.

SENSITIVITY TO CH

The sensitivity of the amount of subsequent in Fig. 4. A median indicates an organ wi
Although the decrease in diameter of the cerebral lobe was small in comparison with the marked changes which occurred in the growth of the cephalic disc, the changes in the relative sizes of the two resulted in the optic anlagen being stretched across the surface of the brain (Fig. 3). The structural collapse of the frontal disc and its failure to differentiate may be a consequence of the direct action of topa, or a combined effect of direct action coupled with the external stresses caused by differential growth of the surrounding tissues.

**SENSITIVITY TO CHEMOSTERILANT**

The sensitivity of the frontal disc in Stomoxys as measured by the amount of subsequent growth following chemosterilant treatment is depicted in Fig. 4. A median inhibitory dose of 1.58 µg during the third instar indicates an organ which is extremely sensitive to topa. The effect of topa
FIG. 4. Effect of teta applied at the beginning of the third instar on the size of the cephalic disc. Each point represents the mean of ten measurements.

on the size of the disc during different stages of the final instar is depicted in Fig. 5. The results emphasize an increase in the final disc area following successively later applications and suggest a decline in sensitivity.

ADULTS DERIVED FROM I AND II INSTAR EXPERIMENTS

As development proceeds, so individual cells of an imaginal disc become progressively more determined into their prospective roles. By applying teta to different stages of this process of determination one can vary the morphology of the resulting adult.

With first and second instar treatments, although considerable mortality occurred at doses greater than 10 ppm (Table II), survivors often emerged with mis-shapen antennae or reduced eyes. The exact transformation of prospective antennal material into leg material noted by Bodenstein and Abdel-Malek [10] in Drosophila using nitrogen mustard was not observed in teta-treated Stomoxys. Variations in the relative size of the arista and its parent segment, enlargement of the entire antennal complex and the occurrence of leg segments were recorded. Antennal mutants were usually able to emerge from the pupal case while insects with damaged compound eyes normally failed and required dissection.

FIG. 5. Effect of teta applied at d each point represents the mean of ten samples.

- day 4
- day 7
- day 9
- day 11

Loss of one or both adult head and also died As the function of the pt puparium when the inac process can easily prey teta may then be explai emerge following incom

ADULTS DERIVED FROM III

Adults which emer somatic mutants of the of the compound eye of of 5 - 10 μg teta during t not alter the shape or a band of abnormally com
The cephalic disc. Each instar is depicted by the relative disc area following the effect of TEPA sensitivity.

ADULTS DERIVED FROM III INSTAR EXPERIMENTS

Adults which emerged from these experiments have so far not included axonic mutants of the types described above. However, the basic structure of the compound eye of this insect was damaged after topical application of 5 - 10 µg TEPA during the latter half of the final instar. Treatments did not alter the shape or size of the eye as damage was confined to a vertical band of abnormally constructed facets on the cornea (Fig. 6).
FIG. 6. Lateral views of the compound eye of Stomoxys calcitrans (L.)

(a) Compound eye of an untreated insect showing the regular arrangement of corneal facets × 32

(b) Compound eye of infected insect showing vertical band of discoid lenses × 32
(b) Compound eye of an insect treated with chemosterilant just before pupation showing a vertical band of disorientated facets × 71
TABLE II. FREQUENCY OF SOMATIC ABERRATIONS IN S. calcitrans expressed as percentages after treatment with TEPA in first and second instars. (A) Emerged Adults. (B) Formed Adults which failed to emerge from pupae

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>(%) Normal</th>
<th>Affected head region</th>
<th>(%) Normal</th>
<th>Affected head region</th>
<th>Postnatal differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antenna</td>
<td>Compound eye</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>35.4</td>
<td>18.0</td>
<td>61.6</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>44.4</td>
<td>100</td>
<td>30</td>
<td>10</td>
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<td>20</td>
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<td>Second instar</td>
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<td>5</td>
<td>60.7</td>
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<td>16.6</td>
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<td>10</td>
<td>35.2</td>
<td>40.9</td>
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Successively later treatments caused the location of this disrupted area to move anteriorly across the eye's surface. In a damaged region uniformly arranged hexagonal facets were replaced first by irregular and then fused facets and finally by complete destruction of the cuticular pattern.

DISCUSSION

The morphological changes which occur during differentiation of the adult Drosophila eye from the larval imaginal disc have been described from the histological and cytological viewpoints by Shatoury [11], Shatoury and Waddington [13] and at the ultrastructural level by Waddington and Perry [15].

In addition Schneider [15] and Gehring [15] have described this process from transplantation experiments and studies in vitro. Recently Frison and Mitchell [16] have outlined a method for isolating large numbers of imaginal discs from Drosophila larvae, allowing for a detailed analysis of the biochemical aspects of differentiation.

The effects of 5-fluorouracil [15], actinomycin D [17], mitomycin C [18], colchicine [19] and nitrogen mustard [20] on development of the compound eye in Drosophila have been described. The authors concluded that compounds known to have a marked effect on cell division disrupt both growth and differentiation of the eye disc. Recently Frison and Knowles [20] provided evidence that actinomycin D inhibited protein synthesis in differentiating imaginal discs of third instar Drosophila larvae.

The general simal and nitrogen mustards are two important agents which cause structural alteration of DNA, actinomycin D and mitomycin C. The mechanism of action of these substances is not clearly understood, but it is generally believed that they inhibit DNA synthesis and modify the cell's processes.
The general similarities between the alkylating mechanisms of aziridines and nitrogen mustards are well documented [21]. The similarity between the major developmental transformation of the arista in tepa-treated Stomoxys and nitrogen-mustard-treated Drosophila also points to a common mode of action. Minor differences in the degree of expression of aristaepida between the two species may be due to the differential responses of the antennal anlagen rather than to differences in the action of the two compounds. There is a more definitive capability of the cephalic disc in particular have been discussed by Gehrig [15]. During the first and second larval instars of the stable fly, cells of the frontal disc become generally determined to form the adult head but local determination into the optic, pollin or antennal regions is not yet fully established. Consequently during this period the relative proportions of the prospective eye or antennal regions in the frontal disc can be altered. Imaginal discs which have been dissected from larvae with disproportionately enlarged antennal regions can presumably be correlated with the fully expressed state of aristaepida in the adult.

In the frontal disc of the first instar further differentiation into groups of retinacular and cone cells of the ommatidia can begin once the size and position of the compound eye have been determined. The final stages of this differentiation which appear to develop and move in a posterior-anterior direction across the eye's surface can be disorganized by tepa. The results also suggest that the chemosterilant is rapidly metabolized as it exerts its action over a very limited period.

In *Aedes aegypti* (L.), another dipterous species, use of microanalytical techniques instead of chemicals [25] has revealed the passage of a factor capable of inducing cell division and ommatidial differentiation in a posterior-anterior direction. Similar processes which begin in the posterior portion and move across the frontal disc in an anterior-posterior direction in *Ephestia* [23] and *Bombyx* [24] suggest that this pattern of differentiation is a general feature of the eye development in many holometabolous insects.

Two possibilities of chemosterilant action may explain the marked suppression of growth observed in imaginal tissue after treatment. Several independent investigations have shown that apholate and other chemosterilants inhibit the production of DNA in insect eggs, including those of *Stomoxys* [25], so that it is highly probable that tepa, a closely related aziridine, will act in a similar manner. Thus the suppression of growth at any stage of imaginal disc development could be explained by the interaction of tepa upon DNA or its formation and its indirect effects on subsequent protein synthesis. A possible analogy is the inhibitory action of actinomycin D on protein synthesis of differentiating imaginal cells of *Drosophila*.

At a different level the amount of growth and differentiation of developing imaginal organs is known to be controlled by a complex balance of hormones which are released by the larval ring gland. Any interference with the normal sequence of synthesis, storage and differential release of these hormones can cause major changes in insect development. The possibility of apholate applied during the larval stage inducing hormonal imbalance in adult *A. aegypti* has been discussed by Sharma and Rai [5].

They concluded that although damage to the nerve fibres and possibly neurosecretory cells of the brain had occurred, there was either considerable repair of the brain tissue, or neurosecretory cells continued to function.

<table>
<thead>
<tr>
<th>head region</th>
<th>Partial compound eye differentiation</th>
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<td>-</td>
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<tr>
<td>-</td>
<td>75</td>
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<td>65.0</td>
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<td>-</td>
<td>50</td>
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<td>64.3</td>
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<tr>
<td>66.3</td>
<td>36.0</td>
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</table>

of this disrupted damaged region by irregular and the cuticular pattern.
correctly but at suboptimal levels. Presumably if any major functional changes in the hormonal output had occurred the majority would have exerted their effects during the larval stage or at metamorphosis.

Observations of the effects of tepa on the neurosecretory cells of the ring gland during larval development of the stable fly have been made and will be described elsewhere. Impairment of the neurosecretory cells has been recorded and these observations support the hypothesis that tepa may influence the insects' hormonal balance.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. C. T. Lewis for his assistance supervising this work and Dr. A. B. Borhovec, of the U.S. Department of Agriculture, for the supply of chemosterilants.

The project was supported by a research grant from the U.K. Ministry of Overseas Development.

REFERENCES


DISCUSSION

E.D. OFFORI: Do you think it is worth pursuing research on the performance of adult Stomoxys calcitrans having deformed compound eyes?

J.S. BADMIN: No, I don't think so. The induction of sterility in the adult stage of this insect is still the most promising area of study.

LA CHIMI-
DE Dacus
DIFFEREN-
CE

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Athènes, Grèce

Abstract — Résumé

CHEMOSTERILISATION DE Dacus

The author attempts to test the effect of Dacus oleae (femelles) on the males by exposing them to the pheromone D. oleae. Les mâles sont les plus sensibles à la chemosterilisation.

LA CHEMOSTERILISATION DES
ENTRE LES DEUX SEXES.

1. Le mode de l'approche de Dacus oleae (femelles) et des males.
2. Les différences entre le système génital des males et des femelles.

INTRODUCTION

Au cours des expériences sur la chimosterilisation de D. oleae, nous avons constaté que les males étaient plus sensibles à la chimosterilisation que les femelles. Nous avons donc cherché à comprendre ce phénomène et à l'utiliser pour contrôler la population d'insectes.

Dans ce travail, nous avons utilisé des méthodes expérimentales et nous avons constaté que la chimosterilisation des males était plus efficace que celle des femelles.

RESULTATS

Au cours de l'étude, nous avons constaté que les males étaient plus sensibles à la chimosterilisation que les femelles.
LA CHIMIOSTERILISATION DES ADULTES 
DE Dacus oleae (Gmelin) PAR LE TEPA 
Différences entre les deux sexes 

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Abstract — Résumé 

CHIMIOSTERILISATION OF ADULT Dacus oleae (Gmelin) WITH TEPA: DIFFERENCE BETWEEN THE SEXES. 
The author attempts to compare the results obtained thus far in a summary study of the genital system of Dacus oleae (Gmelin) with those obtained during chemosterilisation tests with tepa on this insect. The differences in the effect of tepa on the ovaries and testicles seem to be related to the difference in the genital systems of the two sexes. In his conclusion, the author expresses the view that chemosterilisation of Dacus males raises serious problems, while that of the Dacus female is less complicated. 

LA CHIMIOSTERILISATION DES ADULTES DE Dacus oleae (Gmelin) PAR LE TEPA — DIFFERENCES ENTRE LES DEUX SEXES. 
L'auteur tente de rapprocher les données jusqu'ici fournies par une étude sommaire du système génital de Dacus oleae (Gmelin) à celles obtenues au cours des essais de chimiosterilisation de cet insecte par le tepa. Les différences quant à l'effet du tepa sur les ovaires et les testicules semblent être liées aux différences entre le système génital des deux sexes. L'auteur conclut des résultats obtenus que la chimiosterilisation des Dacus mâles pose des problèmes sérieux, tandis que celle des Dacus femelles semble moins complexe. 

INTRODUCTION 

Au cours des expériences entreprises dès la fin de 1966 dans notre laboratoire sur les différents effets du tepa sur les adultes de Dacus oleae, nous nous sommes aperçus que les insectes avaient un comportement différent envers le chimiosterilisant, non seulement par rapport à d'autres insectes mais aussi entre eux (selon leur sexe); ainsi les problèmes de la chimiosterilisation ne paraissent pas être les mêmes pour les adultes mâles et femelles. Nous avons alors émis l'hypothèse que ces différences pourraient être en rapport avec leur cycle génital, jusqu'alors mal connu, c'est pourquoi nous avons entrepris, tout en poursuivant nos essais de chimiosterilisation, un examen sommaire de leur système génital. L'évolution des coocytes dans les ovaires, l'évolution de l'œuf après la ponte (et jusqu'à l'écloration des œufs) et les caryocinéses dans les testicules ont été étudiées par différentes techniques histologiques. 

Dans ce travail, nous avons tenté de rapprocher les données obtenues jusqu'ici concernant l'évolution du système génital des deux sexes de celles obtenues au cours de l'étude des effets du tepa. L'examen du système génital est toujours en cours et la plupart des données que nous rapportons n'ont pas encore été publiées. 

RESULTATS 

Au cours de l'étude du système génital nous avons obtenu quelques résultats que nous résumons ci-dessous.
Cycle génital des femelles

La différenciation de quelques oogonies s'effectue pendant la sortie des adultes [9] et quelquefois à la fin du stade nymphal.

La croissance des oocytes dure au moins quatre jours pour les insectes issus de l'élevage artificiel, et sept jours pour les insectes sauvages. Ce temps constitue la période de provitellogénèse et la seconde la période de vitellogénèse et de la formation de l'ovocyte. Pendant son séjour dans l'ovaire (de la formation de la vésicule germinale jusqu'au passage à travers l'ooviducte), l'œuf possède un noyau en repos (membrane nucléaire et nucléole constamment présents).

Après la ponte ont lieu les deux divisions de maturation (5 à 15 min), la fécondation de l'ovule (environ 30 min), les divisions du cytoplasme (45 min à 5 h) et l'organogénèse (23 h à 70 h) (Fytizas, travail non publié).

La différenciation des oogonies et l'évolution des oocytes sont continues et surviennent pendant toute la vie adulte des femelles; dans le même ovaire, l'évolution de chaque ovariolo est indépendante de celle des autres ovarioles [9].

Cycle génital des mâles

Baccetti et Bairati [1] décrivent la spermatogénèse, qui s'achève chez Dacus oleae avant la sortie des adultes; ce stock de spermatogonides s'épuise avant ou après la seconde spermatogénèse, qui survient 7 à 10 j après la sortie des adultes, et de nouvelles spermatogénèses suivent (une spermatogénèse tous les 6 à 10 j et d'une durée de 3 j) (Fytizas, travail non publié). Après destruction des spermatogonies et des spermatocytes par des rayons gamma [11] ou des substances chimiques [6], les testicules de l'insecte traité sont vidés ou presque au bout de quelques divisions successives. D'après la première des auteurs le stock initial de spermatogonides s'épuise entre le 5e et 11e accouplement, et d'après le second, dès la 6e éjaculation on ne trouve dans les spermatides des femelles fécondées qu'un nombre extrêmement faible de spermatogonides, nombre qui d'ailleurs diminue d'accouplement en accouplement à l'autre et tombe à zéro pour la plupart des individus entre le 9e et le 11e accouplement. Les mêmes auteurs [7, 11], quoique travaillant séparément, ont pu constater que la vigueur sexuelle des Dacus mâles est très élevée; les individus mâles peuvent s'accoupler une fois par jour, et pendant au moins un mois.

A l'encontre de ce qui se passe chez les femelles, les spermatogénèses, qui s'effectuent par étapes, sont synchrones pour une population de mâles donnée; en outre, la caryocinèse est aussi synchronne dans toutes les spermatocytes du même ordre et du même testicule (Fytizas, travail non publié).

Relation entre gamétogénèse et éjaculation des spermatogonides et la ponte

En bloquant l'un des deux mécanismes, nous avons pu constater que le mécanisme de la ponte est indépendant de celui de l'ovogénèse [8] et que celui de la spermatogénèse est indépendant de celui de l'éjaculation [6].

Le rythme de la formation des œufs et celui de la ponte suivent des courbes quasi parallèles. Chez les mâles, aucune relation n'existe entre le rythme de la spermatogénèse et celui des éjaculations.
Parmi les différences apparentes que nous avons pu jusqu'à présent observer entre les deux sexes, résumons celles que nous considérons comme importantes :

- le degré d'évolution des gamétocytes avant que ceux-ci quittent l'organe génital ; chez les femelles ce sont des oocytes de premier ordre immatures, tandis que chez les mâles les spermatozoïdes ont atteint leur forme finale ;

- le rythme de l'évolution des gamétocytes : évolution continue et successive des oocytes dans chaque ovariole, évolution par étapes des spermatozoïdes dans chaque type pour les spermatozoïdes du même ordre et dans le même testicule ;

- parallélisme des courbes de la formation des œufs et de la ponte, absence de relation entre le rythme de la spermatogénèse et les éjaculations.

**DISCUSSION ET CONCLUSIONS**

Les informations que nous avons obtenues jusqu'ici permettraient d'expliquer en partie les différences apparentes lors des essais de chimio-stérilisation des adultes de *Drosophila melanogaster* des deux sexes :

- Le tepa, administré pendant les premiers jours qui suivent la sortie des adultes et à différentes doses, induisait la stérilité totale seulement chez les femelles ; les mâles avaient pendant un temps un certain nombre de spermatozoïdes fertiles, leur stérilité complète s'établissant progressivement, au fur et à mesure que les accomplies devenaient plus nombreux. Une explication de cette différence pourrait être donnée par le degré d'évolution différent des gamétocytes des deux sexes au moment de l'administration du chimio-stérilisant : chez les femelles les gamétocytes sont soit à l'état d'oocytes, soit à celui d'oocytes de premier ordre, gamétocytes en évolution et arrêtés dans leur forme définitive et subsistant plus tard dans les transformations importantes (divisions de maturité) ; chez les mâles, au contraire, les premiers jours et pendant un certain temps, les testicules possèdent des spermatozoïdes complètement formes, gamétases très ou complètement résistantes.

- Quel que soit le temps de l'intervention, les œufs d'une femelle traitée pendant deux heures après l'administration sont stériles [5] ; chez les mâles, l'administration du tepa, même à dose élevée, n'assure pas toujours la stérilité (au moins pour les premiers jours qui suivent le traitement), les résultats dépendant de l'âge de l'insecte. Dans le cas des mâles traités, le fait que la formation des spermatozoïdes est achevée avant le traitement pourrait être la cause de cet échec partiel ; le chimio-stérilisant ne serait efficace que pour les spermatozoïdes et les spermatogonies, ceux qui formeraient plus tard les spermatozoïdes stériles.

- Les différentes zones de gamétocytes dans les ovaires et les testicules présentent une différences quant à la sensibilité envers l'action stérilisante du tepa. Cette différence est bien nette chez les œufs, car les œufs déjà formés sont plus résistants, puis suivent les oogonies et les oocytes n'ayant pas encore été achevée la vitellogénèse. La différence de sensibilité entre les oocytes en croissance et les oogonies nous paraît...
réelle [5], mais la différence entre oocytes en voie de croissance et œufs formés nous paraît assez obscure: le noyau de l’oocyte ne paraît pas subir des modifications appréciables tant que l’oocyte ne quitte pas l’ovaire. Des changements ont bien sûr lieu surtout en ce qui concerne le volume, les constituants chimiques du protoplasme et les parois de l’oocyte. Peut-être sont-ce les parois et la quantité de chimiotestislanant pouvant les traverser qui pourraient fournir une explication: avant et pendant une bonne partie de la vitellogénèse, les cellules folliculaires facilitaient peut-être la pénétration du tepa, transporté par l’hémolymphè; à la fin de la vitellogénèse, le rôle de ce dernier disparaitrait avec elle et le chorion formé créerait une barrière (rôle similaire à celui du puparium empêchant la pénétration de substances comme le tepa) ne laissant au tepa qu’un passage étroit, le micropyle. Si cette supposition se révélait exacte, la différence porterait plutôt sur la quantité pouvant pénétrer dans l’oocyte. En ce qui concerne les mâles, la différence de sensibilité entre les spermatozoïdes et les autres gamétoocytes est marquée. Nous avons signalé lors d’une expérience [6] qu’une différence de sensibilité existait entre les spermatocytes et les spermatozoides, ces derniers étant plus sensibles. Pourtant, des essais ultérieurs ont prouvé le contraire: des examens ont montré que dans le premier cas avait lieu une différenciation des spermatozoides, tandis que dans le second cas nous observations des caryocinèses chez les spermatocystes. On peut donc supposer que l’état des noyaux (au repos, en préparation pour la cinèse et en cinèse) plutôt que l’ordre des spermatozoides ou des spermatogonies, jouait un rôle prépondérant.

Que la sensibilité des différents éléments des testicules change, nous l’avons aussi constaté lorsque le tepa a été administré à des mâles d’âges différents [7], âges correspondant à peu près à un jour avant le commencement de la seconde spermatogénèse et au commencement de celle-ci récente. La précision de la méthode utilisée alors laisse à désirer, étant donné que les insectes pouvaient recevoir le tepa, contenu dans leur nourriture, à l’importante heure du jour de l’intervention, alors que chez d’autres insectes on a prouvé que la sensibilité varie selon l’heure du traitement [2].

– Une autre différence encore apparait, qui concerne la durée de la stérilité induite par le tepa. La stérilité chez les femelles peut être provoquée par une concentration de tepa élevée et par une administration unique; les dégâts que pourrait provoquer cette forte dose priverait la femelle aussi bien d’une descendance viable que des œufs stériles qu’elle aurait pu déposer. Mais pour les Dacus mâles, les doses fortes doivent être évitées, puisque les effets cytotoxiques sur les testicules ne peuvent que nuire à la chimiotestislation [8] et doivent être classés parmi les effets secondaires. D’ailleurs [7], la durée de la stérilité chez les testicules n’a pas été satisfaisante que lorsque la dose (administrée en une seule fois) était proche des doses cytotoxiques. Nous avons obtenu les meilleurs résultats en administrant le tepa à doses faibles, soit tous les 3 j et pendant 2 mois, soit tous les 2 ou 3 j et pendant 10 ou 12 j; dans le premier cas, nous avons tenté de faire coïncider les jours du traitement avec ceux des caryocinèses marquant la nouvelle spermatogénèse; dans le second cas, les traitements répétés à des intervalles de 2 ou 3 j auraient pour effet, d’après Baco, l’accumulation des effets génétiques.
En conclusion nous pouvons constater que les problèmes de la chimio-
stérilisation se posent différemment pour les Dacus mâles et femelles.
En premier lieu, le problème du rapport entre les doses et l’effi-
cacité stérilisante ne se pose vraiment que pour les mâles. La stérilité
permanente chez les femelles peut être induite par une seule adminis-
tration de tepa à une concentration affectant les gamétoocytes les plus ré-
sistants tout en étant inférieure aux doses mortelles, l’utilisation d’une telle
dose ne entraînait pas de conséquences fâcheuses car le facteur de
sécurité est plus que satisfaisant [3]. Mais en ce qui concerne les Dacus
mâles, non seulement nous devons éviter les doses qui pourraient pro-
voquer des effets cytotoxiques ou autres effets secondaires (réduction de la
vigueur sexuelle, réduction du nombre de spermatozoïdes et inactivation
des spermatozoïdes), mais il serait encore souhaitable de déterminer les
facteurs de sécurité entre les doses induisant la stérilité et celles indi-
quant un des effets secondaires, la polygamie des mâles exige pour les
insectes traités non seulement le maintien de la vigueur sexuelle au ni-
veau des insectes normaux, mais aussi la sauvegarde des spermatozoïdes
qui suivaient le traitement.
En second lieu, la notion de «moment judicieux» pour l’intervention a
un sens différent selon qu’on se réfère à l’un ou l’autre sexe. Quel que
soit l’âge des femelles, la présence dans le même ovaire de gamètes
tous les stades possibles d’évolution exclut l’existence d’un temps
d’intervention particulièrement favorable. Pourtant, pour la pratique
agricole, nous admettons que ce problème peut présenter un certain inté-
rêt: l’administration de tepa avant la formation des premiers œufs abou-
trait à une suppression de la fécondité [4] et celle qui suivrait la forma-
tion des œufs serait d’autant plus efficace que le nombre des œufs pondus
jusqu’à 3 h avant l’administration serait plus faible [5]. Le problème
majeur dans le cas des Dacus mâles est de déterminer si la polygamie est
effectuée par étapes et les caryochènes sont synchrones dans
des spermatocytes du même ordre nous permettrait d’intervenir à
un moment déterminé et une détermination de zones de sensibilité ainsi
du changement que subit celle-ci. Pourtant, ce problème se com-
plique par le fait que le changement de sensibilité ne concerne pas la
totalité des gamétoocytes comme c’est le cas chez d’autres insectes, par
exemple les femelles de Cochliomyia hominivorax [10]. Le problème dans
cas des Dacus mâles consiste à suivre l’évolution des différents
gamétoocytes et à déterminer les différentes doses et les différents mo-
ments d’intervention convevables afin d’affecter la matière génétique
de tous les gamétoocytes.
Le cycle génital de l’insecte étudié déterminerait dans un sens la
métode de stérilisation à suivre, et là râle l’importance qu’il acquiert
dans le domaine de la chimio-stérilisation. Les insectes des deux sexes
de la même espèce, comme c’est le cas pour Dacus oleae, n’ont pas
obligatoirement le même cycle génital; on peut constater des différences
appreciables entre femelles du même ordre, comme par exemple celles de
Dacus oleae, de Musca domestica et de Cochliomyia hominivorax; on
peut, enfin, trouver quelques ressemblances entre insectes appartenant
à une espèce et à un sexe différents, comme c’est le cas pour les mâles
de Dacus oleae et les femelles de Musca domestica. Le classement taxo-
nomique des insectes aurait pour l’étude de la stérilisation une importance
quelque peu limitée. Mais il serait fort souhaitable de classer les insectes
auxquels pourrait être appliquée la chimiosstérilisation d’après leur cycle génital; 1’interprétation des expériences de différents chercheurs sur différents insectes serait plus large et la comparaison des données expérimentales plus claire et plus facile.

REFERENCES


DISCUSSION

M.E. TZANAKAKIS: On the basis of your findings, do you consider tepa to be an ineffective means of sterilizing the olive fruit fly in practice?

E. FUTZAS: On the contrary, I think that tepa offers considerable scope for the study of chemosterilization in view of the high safety factor of about 50. The effects of various doses can be studied without the risk of causing death.

M.E. TZANAKAKIS: I should like to put another question if I may. Does your statement that the initial sperm stock is exhausted after the first 7-10 days mean that is exhausted after a certain number of matings by males mating daily?

E. FUTZAS: I agree with you that exhaustion of the initial stock of spermatozoa occurs after a certain number of matings, whatever the interval between two successive matings, the time at which exhaustion of the initial stock occurs is thus in fact independent of the age of the male.
EFECTOS DEL TEPA EN TRES ESPECIES DE TRIATOMINAE

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Abstract — Resumen

EFECTOS DEL TEPA EN TRES ESPECIES DE TRIATOMINAE.

Los autores realizaron experimentaciones de esterilización del teca de Triatominae. Las especies estudiadas fueron Triatoma infestans, Triatoma guasayana y Triatoma patagonica. Los ejemplares de cada especie fueron tepeados en los escarabajos de ninfa V y adultos, mediante técnicas que se detallan en el trabajo.

Las tres especies resultaron sensibles al tratamiento, siendo la más afectada T. patagonica, y en segundo lugar T. guasayana. Los resultados se obtuvieron tepeando las ninñas, logrando porcentajes de esterilidad en mucho de 83% en T. patagonica, 80% en T. guasayana y 78% en T. infestans. La tepeación en adultos tuvo una eficacia menor, logrando porcentajes de esterilidad en los escarabajos y en el orden mencionados del 78, 64 y 59% respectivamente.

No se observó recuperación de la fertilidad en estas experiencias de laboratorio. En la actualidad se está realizando de campo, dado que los resultados observados son prometedores. Sin embargo, es necesario desarrollar un tratamiento adecuado, dado que las especies estudiadas no demuestran ser atraídas por el tepa, mostrando en este sentido un comportamiento distinto al del homóneo pestis.

Con el propósito de probar las posibilidades del empleo de quimioestereilitantes en el control de los Triatominae, hemos ensayado en ellos de tres especies del género Triatoma Laporte: T. infestans (Klug), T. patagonica Del Ponte y T. guasayana Wygodzinsky y Abalos. La primera es el más importante vector de la enfermedad de Chagas en el sur de Sudamérica y las otras dos son las especies periodomésticas más importantes.

Las sustancias empleadas hasta el momento fueron tepa, metapa, afolate y ticas, siendo la primera la que hemos estudiada más completamente y de la que expusimos aquí resultados.

MATERIAL Y MÉTODOS

Cien ejemplares de cada una de las especies fueron separados de sus colonias en el estado de ninfa IV. La mitad de ellos fueron tratados con el producto a los tres días de la eclosión de la ninfa V y los restantes, tres
CUADRO I. PROMEDIOS DE HUEVOS POR HEMBRA, PORCENTAJE DE HUEVOS VIVABLES Y TIEMPO DE INCUBACIÓN EN TRES ESPECIES DE Triatoma

<table>
<thead>
<tr>
<th>Nº de huevos por hembra</th>
<th>% de huevos viables</th>
<th>Tiempo de incubación</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. infestans</td>
<td>240</td>
<td>84%</td>
</tr>
<tr>
<td>T. guasayana</td>
<td>245</td>
<td>94%</td>
</tr>
<tr>
<td>T. patagonica</td>
<td>169</td>
<td>88%</td>
</tr>
</tbody>
</table>

días después de la ecdisis del adulto. Previamente, y fuera de este lote, se habían inyectado veinte ejemplares recién eclosionados de adultos, en la dosis de 0,05 ml de una solución de tepa de 5 mg/ml para verificar si no perjudicaba la vida de los insectos. También se hicieron topicaiones con una solución del producto al 3% para comprobar efectos esterilizantes.

La experiencia que describimos se empleó la topicación en el abdomen de una solución de tepa al 0,1 %, pH 8,2 logrando la alcalinidad mediante el agregado de bicarbonato de sodio. La topicación se realizó en ejemplares anestesiados con éter. La cantidad de tepa aplicada en esta forma fue de aproximadamente 0,1 mg, ya que se empleó 0,25 ml de solución en la topicación completa, en cara ventral y dorsal.

Quince días después de la aplicación en los adultos, estos fueron apareados individualmente con ejemplares del otro sexo no tratados. Los ejemplares tratados en estadio de ninfa V fueron apareados 22 días después de producida la ecdisis a adultos.

Cada pareja fue mantenida en las condiciones generales del criadero, habiéndose elegido igual número de especímenes de idéntico origen y edad como grupo testigo. Las observaciones en cada lote se efectuaron diariamente.

CRITERIOS SOBRE ALGUNOS ASPECTOS

Hemos considerado estéril la hembra incapaz de desovar huevos viables después de copular con un macho no tratado. Con igual criterio, el macho que no fecundó a la hembra no tratada fue considerado estéril.

El número de huevos desovados no ha constituido un criterio de evaluación por cuanto es un dato muy variable según los especímenes. Como dato de interés damos el cuadro I.

Un dato que consideramos importante es la posible modificación de la expectativa de vida de los adultos. En estudios anteriores hemos establecido para estas especies que las hembras adultas tienen una longevidad de 138 días T. infestans, 118 T. guasayana y 96 T. patagonica, mientras que los adultos machos, en el mismo orden, tienen una longevidad de 142, 111 y 94 días, respectivamente.

CUADRO II. PORCIONES TRATADOS CON TSI

<table>
<thead>
<tr>
<th>Trato</th>
<th>Machos</th>
<th>Adulto</th>
<th>Hembra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nº</td>
<td>Nº</td>
<td>Nº</td>
</tr>
<tr>
<td>Adul</td>
<td>36%</td>
<td>36%</td>
<td>36%</td>
</tr>
<tr>
<td>Hembra</td>
<td>36%</td>
<td>36%</td>
<td>36%</td>
</tr>
</tbody>
</table>

RESULTADOS

Los resultados habrán sido:

DISCUSIÓN

Si bien los resultados del tepa sobre las tres especies esterilizan en el 100% empleadas (figura 1). La acción del tepa sobre la ovogénesis y espermatogénesis masculinos,
CUADRO II. PORCENTAJES DE ESTERILIDAD EN TRIATOMINAE TRATADOS CON TEPA Y EN TESTIGOS

<table>
<thead>
<tr>
<th></th>
<th><em>T. infestans</em></th>
<th></th>
<th><em>T. patagonica</em></th>
<th></th>
<th><em>T. gaegayana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tratados</td>
<td>Testigos</td>
<td>Tratados</td>
<td>Testigos</td>
<td>Tratados</td>
</tr>
<tr>
<td><strong>Macho</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adulto</td>
<td>59%</td>
<td>12%</td>
<td>79%</td>
<td>16%</td>
<td>64%</td>
</tr>
<tr>
<td>Niño</td>
<td>79%</td>
<td>13%</td>
<td>89%</td>
<td>10%</td>
<td>82%</td>
</tr>
<tr>
<td><strong>Hembra</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adulto</td>
<td>62%</td>
<td>10%</td>
<td>67%</td>
<td>8%</td>
<td>64%</td>
</tr>
<tr>
<td>Niño</td>
<td>64%</td>
<td>14%</td>
<td>69%</td>
<td>8%</td>
<td>65%</td>
</tr>
</tbody>
</table>

RESULTADOS

Los resultados se exponen en el cuadro II.

DISCUSION

Si bien los resultados obtenidos demuestran una acción esterilizante del tepa sobre las tres especies estudiadas, no se logró en ningún caso la esterilidad en el 100% de los especímenes, al menos en las dosis empleadas (figura 1).

La acción del tepa parece manifestarse en períodos avanzados de la ovogénesis y espermatogénesis, siendo más sensibles los órganos sexuales masculinos.
El no haberse observado recuperación de la fertilidad es un elemento importante, y aunque no se efectuó estudio histológico sistemático, el dato no parece reversible, o si lo es, tiene una lentitud de recuperación que se aproxima al promedio de expectativa de vida del adulto.

El hecho de no haberse observado diferencias significativas en la longevidad de los adultos permite afirmar que el tepa no resulta perjudicial para otros procesos biológicos del insecto estudiado.

En estudios electrorforéticos de la línea de las tres especies no se observaron diferencias significativas de la calidad y cantidad de las proteínas con respecto a las normales que hemos descrito en un trabajo anterior (figura 2).

CONCLUSIONES

Las tres especies resultaron sensibles al tratamiento con tepa, siendo la más afectada T. patagonica y en segundo lugar T. guasayana. Los mejores resultados se obtuvieron topicando las ninñas, lográndose porcentajes de esterilidad en machos de 86% en T. patagonica, 82% en T. guasayana y 78% en T. infestans. La topicación en adultos tuvo una eficacia menor, lográndose porcentajes de esterilidad en las especies y en el orden mencionado del 70, 64 y 56%, respectivamente.

No se observó recuperación de la fertilidad en estas experiencias de laboratorio. En la actualidad se están realizando experiencias de semicampo. Sin embargo, es necesario desarrollar un estratagema adecuado, dado que las especies estudiadas no son atraídas por el tepa, mostrando en este sentido un comportamiento distinto al de R. prolixus con respecto a otro quimioestérilizante.

A. ECONOMOMIC less sterility was induced in R. CARCAVA differentiated cells as on interstitial cells.

D. G. CAMPION: complete sterility?

R. U. CARCAVA: tepa, metapa, apholes to the effective amount (terminales).

One fact that I was to be more sensitive sterile, while others widely from one indiv.

D. S. GROCH: (o) to repair lesions inducible differences in gene such an ability, a re demonstration this later.

L. E. LACHANCE male to male encounter little variability in their high doses of tepa, presented. One should and the possible use (h)

D. S. GROCH: I is variable, a principle.

J. C. GOMEZ-NU apparently equal dose advisable to consider searching for complex...
BIBLIOGRAFIA

BIBLIOGRAFIA


REIDEN, G.S., EMILTE, B.J., Chemosterilant studies with the German cockroach, Pla. Entom., 463 (1960) 229-234.


DISCUSSION

A. ECONOMOPoulos: How do you explain the fact that in your work less sterility was induced when adults were treated?

R. U. CARCAVALLO: I think the reason is that tepa acts on less differentiated cells and also, according to the results of recent studies, on interstitial cells.

D. G. CAMPION: Why did you not use higher doses of tepa to obtain complete sterility?

R. U. CARCAVALLO: We were conducting a comparative study with tepa, metepa, apholate and thiourea, and adopted a standard dose equal to the effective amount of the most active of these four chemosterilants (thiourea).

One facet that I am unable to explain is why some individuals appear to be more sensitive than others to certain chemosterilants. Some remain sterile, while others recover. Even at the dose limit, behaviour has varied widely from one individual to another. I wonder whether anyone here has an explanation for this?

D. S. GROSCH: One suggestion is that insects may differ in their ability to repair lesions induced by chemical agents. In other words, because of differences in genetic make-up, individuals may or may not possess such an ability. A recent thesis by A. C. Hoffman, of my laboratory, demonstrates this for a different agent.

I. E. LaCHANCE: With regard to the variability in sterility from male to male encountered in studies on Triatoma infestans, we found very little variability in thousands of treated male house flies either at moderate or high doses of tepa. I would therefore disagree with the explanation just presented. One should first eliminate variation in the dose to each male and the possible use of a few non-virgin females.

D. S. GROSCH: Mr. LaChance is convinced that biological material is variable, a principle to which I subscribe.

J. C. GOMEZ-NUÑEZ: With respect to variations in the response to apparently equal doses of chemosterilants by individual triatomids, it seems advisable to consider the possibility of human error in the dosage before searching for complex biological reasons.
F.M. WIENDL: Mr. Carcavallo, why did you use ether and not carbon dioxide for anaesthesia, in view of the fact that it probably has an influence on longevity?

R.U. CARCAVALLO: Ether is practical and harmless at the doses we use. I don't believe that it affects longevity, and it is used as a matter of routine at our laboratory. The insects are anaesthetized with ether, for example, to prevent their escape when the flasks containing them are cleaned.

AUTOSTERILIZATION OF TSETSE

The difficulties at present involving the sterile-male tech method involves sterilising flies in various ways, it would appear the absence of a suitable batch of Glossina (e.g. tsetse) or who suggested in this paper a balance along with domesticated pets in pick up the chemical and it is also discussed.

INTRODUCTION

The possibility of tsetse population has of the main initial diff the flies in large numbers of the very low rate of parous; the female p of 10 to 15 larvae in k program is thus limit reproduction.

In recent years a which may be effected adults may be exposed to a fine spray of sterile natural population. A flies are sterilized w the model suggested population. Such an aing large numbers of difficulty of breeding Dame and Schmidt [5] of chemosterilants pr only the target species attractant is as yet kn sterilant for tsetse flies of Glossina or some t purpose.
AUTOSTERILIZATION OF TSETSE FLIES
A model for use with chemosterilants

E. D. OFOSU
Animal Research Institute,
Council for Scientific and Industrial Research,
Achimota, Ghana

Abstract

AUTOSTERILIZATION OF TSETSE FLIES. A MODEL FOR USE WITH CHEMOSTERILANTS.

The difficulties at present encountered in mass-breeding tsetse flies for use in eradication programs involving the sterile-male technique demand that the problem be tackled from several other angles. One method involves sterilizing flies in the natural population (autosterilization). While this could be effected in various ways, it would appear that with chemical sterilization, an attractant would be required. In the absence of a suitable chemical attractant for use with tsetse flies, it is suggested that both animals of Glossina (e.g., cattle) or other physical attractant such as a moving vehicle be employed. In the model suggested in this paper a balloon is dosed with an "over-all" impregnated with chemosterilant and driven along well demarcated paths in a tsetse-infested area. Flies that make contact with the "over-all" then pick up the chemical and, it is hoped, become sterilized. Some of the pros and cons of this approach are also discussed.

INTRODUCTION

The possibility of applying the sterile-male technique for eradicating tsetse populations has been discussed by several authors [1 - 5]. One of the main initial difficulties that need to be overcome is that of breeding the flies in large numbers. This difficulty arises primarily because of the very low rate of reproduction of tsetse flies. The insect is larviporous; the female produces only one larva every 10 days and a total of 10 to 15 larvae in her lifetime. The success of a mass production program is thus limited, among other factors, by this slow rate of reproduction.

In recent years attention has been directed to autosterilization which may be effected by several methods. With chemicals, emerging adults may be exposed to a treated surface or be made to pass through a fine spray of sterilant before the flies are allowed to mix with the natural population. Alternatively pupae may be treated so that emerging flies are sterilized when they come into contact with their own puparia. The model suggested in this paper involves sterilizing flies in the natural population. Such an approach would eliminate the risk involved in releasing large numbers of laboratory-bred flies as well as help to by-pass the difficulty of breeding and maintaining Glossina under laboratory conditions. Dame and Schmidt [5] have pointed out that owing to the mutagenic activity of chemosterilants presently available, it is important to ensure that only the target species is affected. In view of the fact that no chemical attractant is as yet known that could be used effectively with a chemosterilant for tsetse flies, the present paper suggests that the host animals of Glossina or some other physical 'attractant' be employed for the purpose.
The model is based upon our experience in the field, namely that

(i) tsetse flies are attracted to moving objects—animals, humans, or vehicles, and
(ii) in tsetse survey work, definite paths (fly rounds) are often marked out and followed at regular intervals for the purpose of estimating tsetse population densities.

PROCEDURE

The target area is divided into patches (fly resting haunts) about 100 yards by 100 yards, separated by reasonably wide paths (fly round), as shown in Fig. 1. A tame cow or bullock is procured to serve as both an attractant and chemosterilant applicator. The bullock is donned with an ‘over-all’ (Fig. 2), preferably of a dull brown or black coloration. The ‘over-all’ is then smeared or otherwise treated evenly with chemosterilant. To prevent seepage through the ‘over-all’, a non-absorbent material may be used. The bullock thus treated, is driven slowly along the paths and close to the fly haunts (Fig. 1). As the flies alight and begin to probe through the ‘over-all’, they pick up chemosterilant via the legs and proboscis, as well as other parts of the body that may come into contact with the sterilant.

FIG. 1. Model ‘fly-round’ along which a bullock may be driven.

FIG. 2. Bullock in ‘over-all’.

DISCUSSION

It is our experience that a treated surface (in a treated surface flies would not produce high mo quantity of chemoster [5] reported no sign. G. moritans were ex with tepa or metapa a model, it is unlikely that surface continuous, or at least successful.

The approach involving ages with the result in complete site sterilized. Thorough doses for flies of adult male G. moritans obtained complete site Mules exposed for 15 therefore that the tim cating factor.

Chemosterilants of atmosphere. Thus, a sterility in the first 3 effectiveness of if the advantages of ti insects are sterilized
BULLOCK 'OVER-ALL'
IMPEregnATED WITH
CHEMSTERILANT

FIG. 2. Bullock in 'over-all'. The animal serves as both an attractant for flies and chemosterilant applicator.

DISCUSSION

It is our experience that tsetse flies make several 'landings' on a host animal (or moving vehicle) before settling down to take a blood meal. Thus, the probability of a fly picking up enough sterilant from a treated surface (in this case the bullock's 'over-all') is quite high.

It is possible also that by making several contacts with the treated surface flies would acquire an overdose of sterilant, which could therefore produce high mortality. This may be offset by applying a measured quantity of chemosterilant evenly on the 'over-all'. Dame and Schmidt [5] reported no significant shortening of life span when adult male G. morsitans were exposed for 30-60 min to a glass surface treated with tepa or metepa at the rate of 10mg/m². According to the present model, it is unlikely that a fly would remain in contact with the treated surface continuously for over an hour, especially if it is unable to feed or at least successfully probe through the 'over-all'.

The approach involves also the possibility of sterilizing flies of varying ages with the same dose of chemical. Theoretically this could result in complete sterility of some flies while others become only partially sterilized. Thorough laboratory investigation of the minimum sterilizing doses for flies of different ages is therefore a necessary prerequisite.

In their experiment with tepa and metepa Dame and Ford [6] exposed adult male G. morsitans of varying ages to treated glass surface and obtained complete sterility when the exposure time was 30 or 60 min. Males exposed for 15 min, however, recovered fertility. It would appear therefore that the time of exposure and not the age of the flies might be a limiting factor.

Chemosterilants become degraded upon prolonged exposure to the atmosphere. Thus, although Dame and Schmidt [5] achieved complete sterility in the first 3 months with tepa applied to pupae of G. morsitans, the effectiveness of the chemosterilant declined rapidly thereafter. One of the advantages of the present approach therefore would be that the insects are sterilized without the necessity of exposing the chemical
in the field for too long a time. It would be necessary, however, to repeat the operation at regular and frequent intervals.

It is conceivable that a male fly sterilized by the present method could, in turn, sterilize several females with which it may come into contact during mating. The effectiveness of this (secondary) sterilization by contact would of course depend, among other things, upon the quantity of sterilant picked up by the male at the time it alighted on the treated surface.

The necessity for selecting chemosterilants that would only affect the target species has been pointed out [5]. By using the present method, the host animal of the tsetse is employed as an attractant in order to minimize the possibility of affecting other insects in the environment. Field observations, however, show that a number of other species, especially among the Diptera, are attracted to cattle. Most of these, including tabanids, face flies, horn flies, and stable flies, are either nuisance flies or pests involved in the mechanical transmission of trypanosomiasis in cattle. Thus, even if the present method is not entirely selective against tsetse flies, it is obvious that most of the other insects that may be affected are in fact undesirable species that need to be eradicated.

REFERENCES


DISCUSSION

W.F. BALDWIN: May I ask Mr. Offori if he has considered the distances over which the flies might be expected to respond to a moving object? It seems to me that this would be a very important consideration in developing his model for the auto sterilization of tsetse flies.

E.D. OFFORI: Tsetse flies use the olfactory followed by the visual sense to locate their host. If we drive the bullock or the treated vehicle close enough to the vegetation in which the flies were resting, the necessary response will be elicited. This has in fact been demonstrated in the field in Ghana for one or two species by Chapman.

P. MACHILL: Since tsetse flies normally follow a moving animal or any moving object, is it not of some advantage to give the test animal certain types of stimulants or irritants to make it more mobile than usual?

E.D. OFFORI: moves slowly. We can (about 5 miles/h) than

P. MACHILL: Of weather. Presumably, the test area during dry off by rain. Unfortunately, are less mobile and no any comments about... E.D. OFFORI: which can drive this it is immaterial what P. MACHILL: Le instead of cows, which but also with their ow... E.D. OFFORI: I appreciate that as one to control the method if you were to apply it to a tame bullock or, by G.R. SETHI: Yo portion of the body with chemosterilant, to give more treated... E.D. OFFORI: I out, but I think your task the temperature is rat... E.D. OFFORI: The many problems we 'model', it will not be too long a time, so that R.C. BUSHLAND area using chemosteril... have your bait animals E.D. OFFORI: I bails or a moving Lear to attract large number Ghana, despite the fact vegetation.

A. MEWS: Am I the majority of flies at this have any effect on E.D. OFFORI: That preliminary studies of we observed that the n to 1. The effect on my it is the males that we
E.D. OFFORD: In point of fact it is better if the animal (or vehicle) moves slowly. We catch more flies when the vehicle is driven slowly (about 5 miles/h) than when it is moving fast.

P. MACHILLI: One other important factor would be the effect of the weather. Presumably the test animals would have to be released into the test area during dry weather so that the chemosterilant is not washed off by rain. Unfortunately, dry weather is the time when the animals are less mobile and more likely to be resting in the shade. Have you any comments about this?

E.D. OFFORD: Yes. In this model, I propose to use a tame bullock which I can drive around the area. The bullock will attract flies, which will receive chemosterilant from the animal and return to the wild population. It is immaterial whether the wild host animals are resting in the shade or not.

P. MACHILLI: Lastly, have you thought about using wild animals instead of cows, which would have to contend not only with the over-all survival, but also with their own survival?

E.D. OFFORD: I don't want to use wild animals. I am sure you appreciate that as chemosterilants are dangerous, we should be able to control the method of application. You would not have this control if you were to apply the chemical to a wild animal. That is why I prefer a tame bullock or, better still, a vehicle.

G.R. SETHI: Your Fig. 2 gives the impression that only the upper portion of the body of the animal is to be covered with material treated with chemosterilant. Would it not be better to cover the whole body to give more treated surface area with which the flies can come into contact?

E.D. OFFORD: The final details of the over-all have not been worked out, but I think your suggestion is a good one.

G.R. SETHI: You propose to try this technique in Africa, where the temperature is rather high in the field. Work on persistent insecticides shows that the loss of insecticide is considerably greater at high temperatures. Wind velocity has also been found to affect persistence. Would this phenomenon not have an adverse effect on your trials?

E.D. OFFORD: That is quite possible, and I admit that it is one of the many problems we have to contend with. However, according to my 'model', it will not be necessary to expose the chemical in the field for too long a time, so that the danger you refer to will be minimized.

R.C. BUSHLAND: If you wish to sterilize 90% of the flies in a given area using chemosterilant-treated animals, will it not be necessary to have your bait animals outnumber the wild animals at a ratio of 9 to 1?

E.D. OFFORD: I don't think this will be necessary. Using human baits or a moving Landrover vehicle, we were able on many occasions to attract large numbers of flies in the Mole Game Reserve in Northern Ghana, despite the fact that wild host animals were present in the nearby vegetation.

A. MEWS: Am I right in thinking that in a number of these species, the majority of flies attracted to a moving object would be males? Would this have any effect on your theoretical model?

E.D. OFFORD: The answer to your first question is yes. During preliminary studies of Glossina morsitans in the Mole Game Reserve we observed that the males outnumbered the females in the ratio of 20 to 1. The effect on my 'model' would be advantageous because, after all, it is the males that we wish to sterilize.
EFFECTS ON 4th- \textit{A. Oncopeltu} 

Development of tretamine 

A. P. ECONO

Department of Biology 
University of California, Berkeley, Cal

Abstract

EFFECTS OF TRETAMINE ON 4th-INSTAR EGGs FROM TRETAMINE-TREA

One microgram of tretamine, a 4th-instar instar of Oncopeltus, in the next mouth, similar application results in 60% but a small part at stages with spermatogenesis and formation. Application on 4th instars later-treated male nymphs (males) 
The early-treated 4th-instar nymphs adults and many of them survive effect of tretamine.

One-day-old female bugs dissected out after 5 and 25 days had ovaries with small oocytes on the second day of adult life (variable size, the smaller shown to the morphoytes.

INTRODUCTION

Tretamine was for males when treated at blastocyst stage; a few days after treatment the mature eggs were divided by a low or diminished fertile stage. Already started, the mature eggs (devoid of any arrest) in the productivc ovaries produced in experimenters a few days after treatment suffered minor damage and found a similar significant metapata-treated \textit{O. fasciatus}.

* Present address; “Domic
EFFECTS OF TRETAMINE
ON 4th- AND 5th-INSTAR
Oncopeltus fasciatus NYMPHS

Development of eggs from
tretamine-treated females

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Abstract

EFFECTS OF TRETAMINE ON 4th- AND 5th-INSTAR Oncopeltus fasciatus NYMPHS: DEVELOPMENT OF EGGS FROM TRETAMINE-TREATED FEMALES.

One microgram of tretamine, topically applied in acetone solution at the beginning of the 4th- and 5th-nymphal instars of Oncopeltus fasciatus (Dallas) (Heteroptera; Lygaeidae), prevented almost completely the next molting; similar application toward the end of the 4th instar permitted the majority of the nymphs to molt into 5ths but a small percentage of them succeeded in running into abnormally adults. All these treatments, at stages with spermatogonia and spermatocytes present but no sperm produced yet, did not prevent sperm formation. Applications on mid- or late-5th instar nymphs had no apparent effect on adult molting and the later-treated male nymphs (more sperm present) showed, as adults, less mating activity and more sterility. The early-treated 5th instar nymphs had a reduced weight at the time when they should start molting into adults and many of them remained as abnormal 5ths for an unusually long period; most died of an apparent effort to molt.

One-day-old female bugs were treated topically with 1 μg/mouse of tretamine and their ovaries were dissected out after 5 and 55 days. At day 5 most of the ovaries contained no apparent oocytes and very few had ovaries with small oocytes; at day 55 several ovaries showed various stages of recovery. Females treated on the second day of adult life (oocytes not fully developed yet) and mated with normal males, laid eggs of variable size, the smaller showing higher embryonic death. The striking reduction in egg size suggests injury to the neurosecretory system.

INTRODUCTION

Tretamine was found highly effective in sterilizing Oncopeltus fasciatus males when treated as adults [1]. Subsequent work showed that female bugs were also sterilized by tretamine. When young females were treated, a low or diminished fecundity was observed; treatment at a later stage, egg laying already started, resulted in a drastic reduction of egg viability for the mature eggs (developed and stored before tretamine application) and an arrest in the production of new eggs [2]. Eggs of small size were always produced in experiments with chemonsterilized females; they were detected a few days after treatment and were probably produced by females which suffered minor damage and had started recovering. Lawson and Ball [3] found a similar significant reduction in the number of eggs produced by metopa-treated O. fasciatus females.

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Sperm formation in *Q. fasciatus* males starts at the beginning of the 5th nymphal instar, and by adult day 1, half of the testes volume is occupied by sperm [2]. Synthetic compounds with juvenile hormone activity (6th-instar nymphs produced) did not alter the sequence of sperm production and the same was true when testes from very young 5th-instar nymphs or late 4th (sperm not produced yet) were transplanted into adult male or female milkweed bugs.

This work was designed to investigate the effects of tretamine on *Q. fasciatus* nymphs as well as to study the development of small and normal-size eggs from tretamine-treated females.

**MATERIALS AND METHODS**

The polyfunctional aziridine tretamine (2, 4, 6-tris (1-aziridinyl)-s-triazine) was provided by J. S. Bowman (Agricultural Division, American Cyanamid Co., Princeton, N. J., U. S. A.). Solutions of 1 µg/d in acetone were prepared just before topical application. The Hamilton repeating dispenser was used for topical application of one 1-µl droplet on the ventral thorax, between the legs. The insects were anesthetized with CO₂ before treatment for easier handling. The bugs used were of the CS-18 and CS-19 strains (18 and 19 generations of consecutive rearing on a mixture of cashew nuts and sunflower seeds). Whenever bugs were needed for experimentation, colony cages containing bugs of a stage of development preceding the one needed were transferred into an incubator with a constant temperature of 30°C. At this temperature development is accelerated and the experimental time is shortened. Whenever nymphs were treated they were from both sexes and in a 1:1 sex ratio. The colony bugs were kept at room temperature (20-25°C) in the plastic cages developed in the Department of Entomology, University of California (Gordon and Jao, unpublished data).

For dissections the living females were pinned on their backs to a paraffin-bottom glass dissecting dish covered with an insect saline (6.5g NaCl, 0.25g KCl, 0.25g CaCl₂ and 0.25g NaHCO₃ in 1 litre of distilled water). The female reproductive systems were studied or photographed soon after dissection in the saline.

Egg development after tretamine treatment was studied in females that were treated on day 2 of adult life. About 40 treated females were mixed 5 days after treatment with an equal number of normal males of similar age. Eggs laid during the 7th and 8th days were discarded and from then on the eggs were harvested daily, from the special egg-laying holes covered with cheesecloth, and held in EP-14 polyethylene cups covered with EP-16 ones (Protective Closures Incorporation, 2207 Elmwood Avenue, Buffalo, New York 14216). The eggs were kept at 30°C for a period of 8 days. The control cage had also 40 bugs from each sex at the beginning.

Only a random sample of 40-80 control eggs were kept daily for examination. Control eggs produced during the first or last days of the second period were not used, to avoid the age effect described by Richards and Kolderie [4]. These workers found that very early and very late in the second period *Q. fasciatus* females lay fewer eggs that weigh less, take longer to develop and give lower hatching percentages.

**TABLE I. PERCENT NYMPHS THAT SUCH AFTER TOPICAL APPLICATION**

<table>
<thead>
<tr>
<th>Treated insects</th>
<th>0 - 24-hour-old 4th-instar nymphs</th>
<th>24-hour-old 5th-instar nymphs</th>
<th>3-day-old 5th-instar nymphs</th>
<th>6-day-old 6th-instar nymphs</th>
<th>Control A</th>
<th>Control B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a No controls for the ear experiments with com were treated topically b Mostly weak and absc d 0 - 24-hour-old 5th-instar nymphs e 6-day-old 6th-instar nymphs f Control nymphs not treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE II. AVERAGE NYMPHS TREATED**

<table>
<thead>
<tr>
<th>Insects</th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a 10 in parenthesis</td>
</tr>
</tbody>
</table>

Eggs recorded as distended for the greater Lawton and Ball [3] in a non-cellular structure eggs from virgin *Q. fasciatus* and dull appearance &
TABLE I. PERCENTAGES OF 4th- AND 5th-INSTAR *O. fasciatus NPMPHS THAT SUCCEEDED IN MOUTHING INTO 5ths AND ADULTS AFTER TOPICAL APPLICATION OF 1 μG OF TRETAMINE

<table>
<thead>
<tr>
<th>Treated Insects</th>
<th>No. of insects</th>
<th>Percent that moulted to 5ths</th>
<th>Percent that ended up as adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 - 24-hour-old</td>
<td>40</td>
<td>7.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3</td>
</tr>
<tr>
<td>4th-instar nymphs&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late 4th-instar nymphs</td>
<td>50</td>
<td>70.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 - 24-hour-old 5th-instar nymphs</td>
<td>144</td>
<td>0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>6 - 4-day-old 5th-instar nymphs</td>
<td>55</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>6 - 7-day-old 5th-instar nymphs</td>
<td>30</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>Control A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25</td>
<td>92.3</td>
<td></td>
</tr>
<tr>
<td>Control B&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25</td>
<td>93.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> No controls for the early and late 4th-instar treatments were undertaken. However, in other experiments with compounds having juvenile hormone activity, early and late 4th-instar nymphs were treated topically with 1 μg of acetone; more than 80% of the treated nymphs moulted into 5ths and adults respectively.

<sup>b</sup> Mostly weak and abnormal insects.

<sup>c</sup> 6 - 24-hour-old 5th-instar nymphs treated with 1 μg of acetone topically.

<sup>d</sup> Control nymphs not treated with acetone.

TABLE II. AVERAGE BODY WEIGHT IN mg OF 5th-INSTAR *O. fasciatus NPMPHS TREATED WITH 1 μG OF TRETAMINE TOPICALLY

The nymphs were treated as 6 - 24-hour-old 5ths and weighed 6 days later (just before moult into adults)

<table>
<thead>
<tr>
<th>Insects</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>37.3 (36)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.4 (44)</td>
</tr>
<tr>
<td>Control</td>
<td>49.6 (36)</td>
<td>41.3 (25)</td>
</tr>
</tbody>
</table>

<sup>a</sup> In parentheses the number of insects used.

Eggs recorded as presumably fertilized in Table III remained fully distended for the greater part of all of the observation period of 6 days. Lawson and Ball [5] reported that fertilized *O. fasciatus* eggs develop a non-cellular structure at the surface; they did not find the above layer in eggs from virgin *O. fasciatus* females, and these eggs exhibited a shrunken and dull appearance soon after oviposition.
### TABLE III. REPRODUCTION VALUES* IN NORMAL MILKWEED BUG FEMALES CAGED WITH MALES TREATED AS 4th- AND 6th-INSTAR NYPHES WITH 1 mg OF TRETAMINE

<table>
<thead>
<tr>
<th>Treated lines</th>
<th>Total eggs produced</th>
<th>TPFE</th>
<th>TN</th>
<th>PFT</th>
<th>PV1</th>
<th>PVCH</th>
<th>MDAYS</th>
<th>FDAYS</th>
<th>MAT</th>
<th>E/ED</th>
<th>PM</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late 4th-instar male nymphs</td>
<td>362</td>
<td>96</td>
<td>0</td>
<td>25.0</td>
<td>0.0</td>
<td>0.0</td>
<td>165</td>
<td>48</td>
<td>0</td>
<td>7.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3 - 4-day-old 5th-instar male nymphs</td>
<td>134</td>
<td>1063</td>
<td>502</td>
<td>92.0</td>
<td>62.0</td>
<td>48.7</td>
<td>84</td>
<td>61</td>
<td>11</td>
<td>18.9</td>
<td>13.1</td>
<td>18.0</td>
</tr>
<tr>
<td>6 - 7-day-old 6th-instar male nymphs</td>
<td>686</td>
<td>367</td>
<td>137</td>
<td>57.9</td>
<td>38.5</td>
<td>22.9</td>
<td>84</td>
<td>71</td>
<td>5</td>
<td>8.7</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Control</td>
<td>4846</td>
<td>4873</td>
<td>1908</td>
<td>98.3</td>
<td>90.8</td>
<td>89.4</td>
<td>416</td>
<td>350</td>
<td>150</td>
<td>12.4</td>
<td>38.1</td>
<td>42.9</td>
</tr>
</tbody>
</table>

* The abbreviations used have the following meaning: TPFE total number of eggs recorded as presumably fertilized, TN total number of nymphs that hatched, PFT percentage of nymphs that hatched, PV1 percentage of total eggs laid that hatched into nymphs, PVCH percentage of total eggs laid that hatched into nymphs, MDAYS male-days during the experimental period, FDAYS female-days during the experiment, MAT total number of mating observed during the experiment, E/ED total number of eggs divided by the number of female-days, PM percentage of males observed mating, PF percentage of females observed mating.

### RESULTS AND DISCUSSION

The results show that treatment with 1 mg of tretamine had a detrimental effect on the reproduction of milkweed bugs. The table above indicates that the survival rate of nymphs was significantly reduced, as reflected by the lower TPFE and TDYS values compared to the control. The percentage of eggs that hatched into nymphs, as well as the number of male and female-days during the experiment, were also lower in the treated groups, indicating reduced mating and oviposition rates. These findings suggest that tretamine might be a promising candidate for control measures targeting milkweed bugs.
RESULTS AND DISCUSSION

Nymphal treatments

Table I shows that when very young 4th- and 5th-instar nymphs were treated with 1 μg of tretamine the next moulting was prevented in almost all nymphs. The majority of late 4th-instar nymphs treated with tretamine succeeded in moulting into 5th instars and a considerable percentage finally moulted into adults. A total of 82% of the nymphs treated as mid-5th instars turned into adults and the late-5th-treated moults in percentages not different from the controls. Fifth-instar nymphs treated at the beginning of the instar had a reduced weight at the end of a normal instar period (Table II). Several nymphs treated as early 4ths or late 4ths remained 4ths and 5ths, respectively, for an unusually long period. The phenomenon appeared more striking (because of lower mortality) in nymphs treated as early 5ths. Thus, out of 144 treated nymphs 30 were found surviving as abnormal 14-day-old 5ths and 20 were alive 20 days after treatment. The 5th-instar usually lasts 6 - 7 days at 30°C. In a control test only 12 of 112 5th-instar nymphs remained 5ths for up to 15 days; this could be attributed to some hormonal imbalance caused by genetic variability.

Since the same amount of tretamine was applied on all nymphs of Table I, the earlier stages received a much greater dose per unit body weight. The average body weight of a 0 - 24-hour-old 4th-instar nymph was found [2] to be 3.3 mg, of a 0 - 24-hour-old 5th-instar nymph 23.3 mg and of a 0 - 24-hour-old adult 49.6 mg. Seeing that there is no significant weight difference between a nymph at the end of any instar and the same nymph at the very beginning of the next instar, one can approximate the applied quantities of tretamine to 18, 40, 40 and 20 μg Kg for the early 4th, late 4th, early 5th and late 5th-instar nymphs respectively. Thus one can explain the increased mortality in the early-4th-treated nymphs where only 6 (3 4ths and 3 5ths) out of 40 were surviving 12 days after treatment. In the late-4th-treated nymphs 27 (12 5ths and 15 adults) out of 50 were surviving 12 days after treatment. The fact that the majority of the 4th-instar nymphs treated at the end of the instar succeeded in moulting into 5ths and many of them ended up as adults but only one out of 144 5ths treated at the beginning of the instar moulting into an adult, indicates a special chemosterilant sensitivity at the beginning of the instar. Harwalkar and Naif [5], working on another hemipterous insect, Dydervus koenigii (Pict.), found that X-irradiation of an instar during its late phase did not affect its next moulting; irradiation at the early stage did affect the next moulting. They suggested that treatment at a late stage is not very critical for the approaching moulting since the epidermal cells have already divided.

A similar situation could be the case with the chemosterilants that inhibit mitosis. Kuzin et al. [6] showed that puptation in g-irradiated larvae of Ephesia kühniella is not inhibited because of a damage to the DNA of the hypodermal but because of the absence of the moulting hormone, ecdysone. During the 5th instar of O. fasciatus the amount of ecdysone/g of bug is much higher between days 1 to 6 than days 0 and 7, no ecdysone was found on the day of adult eclosion, and a low level was found in the subsequent days [7]. Therefore, if tretamine damages the neurosecretory cells, as g-irradiation seems to do in Ephesia kühniella, an early treatment in the 5th instar could deprive the insect of the hormone level necessary for
moulting. In all nymphal treatments abnormal forms were observed towards
the end of the instar. These forms looked like nymphs in an effort to
moulte with wing pads abnormally developing and the old cuticle never cast
off successfully; all died before moulting. The majority of these abnormali-
ties were found in nymphs treated as young 5ths. Very few were observed
in other treatments, and in nymphs treated as late 4ths the phenomenon
appeared towards the end of the next instar. Partial damage to the dividing
cells or inadequate quantities of ecdysone could be the cause of these
irregularities.

Adult males treated from the nymphal treatments (Table I) were
caged with normal females of similar age. For 15 days the eggs were
collected and examined to evaluate the male sterility. The control data
represent the 20 1st days from a typical control cage used at that time for
the main sterility experiments [2]. In all cases 5 - 8-day-old males were
caged with females of similar age. Table III shows that males treated as
late 4-th instar nymphs gave zero observed mating activity during the
15-day period that they were caged with normal females. These males
were weak insects weighing 36, 6 mg each, on the average, instead of 50 mg
which is the average body weight of a normal male. At the end of the
15-day period the surviving males were opened and large quantities of
sperm were detected in the testes and the seminal vesicles. Thus, treat-
ment at a stage with no sperm present yet, does not seem to prevent its
formation. The substantial number of eggs recorded as fertile, even without
any observed mating activity, indicates that the discrimination of eggs into
fertile and infertile soon after oviposition is not highly accurate (something
already seen in nymphs work with O. fasciatus [2]).

Males treated in the middle of the 5th instar gave double mating activity
compared with those treated at the end of the instar. Fertility, viability
and hatchability were also much higher in the case where males were
treated as mid-5ths. The much larger number of eggs per female-day in the
cage with mid-5th-treated males is probably the result of a more intense
mating activity. Gordon and Loher [8] showed that mating activation is the
dominant factor controlling egg production in the CS strain. On the other
hand, the lower egg production rate in the control cage is probably connected
with the larger number of insects in the cage [2].

The observed male mating activity in both mid- and late-5th-instar-
treated males was much lower than the control and the phenomenon is
especially striking in the late-5th-treated. Seeing that in other experiments,
with males treated as young adults with 20 mg/kg of tretamine and with cage
density well above the 14 insects, the male mating activity for the 1st
20 experimental days was above 20-25% (percent males mated) [2], one
concludes that nymphal treatments are more harmful to the vigour and male
mating drive. In both mid- and late-5th-instar treatments the first fertilized
eggs appeared 10 - 12 days after treatment and fertility, viability and hatch-
ability were constantly higher in the mid-5th treatment. In both treatments,
viability and hatchability are considerably higher than in experiments with
males treated as young adults with 20 mg/kg of tretamine [2]. Thus, the
data suggest that nymphal treatments caused more damage to the male
mating vigour and less sterility. However, the small insect numbers used in
the above experiments do not allow any firm conclusion on mating activity
and sterility in adults treated as nymphs with tretamine. A preferential
major effect of tretamine on mature spermatoroa could explain the lower

![Image](image-url)

Fig. 1. Ovaries from a 6-day-

sterility induced in a
late-5th-instar male
Fahmy and Fahmy [9]
affected by tretamine
and spermatids are fi

Adult treatments

Figures 1 - 3 pre-
treated female bugs,
development. Upon 1
appeared in some of 1
ovarioles full of grow
ova; ovaries with no
similar to Fig. 2. Ye
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nutritive cells or bod
were observed towards 48h in an effort to
old cuticle never cast
ity of these abnormality.
very few were observed
48h the phenomenon
age to the dividing
cause of these
ments (Table 1) were
ays the eggs were
The control data
used at that time for
male males were
was treated as
activity during the
ses. These males
age, instead of 50 mg
the end of the
large quantities of
cicles. Thus, treat-
seem to prevent its
fertile, even without
mination of eggs into
accurate (something
double mating activity
Fertility, viability
males were
per female-day in the
faint of a more intense
ating activation is the
strain. On the other
re is probably connected
and late-5th-instar-
phenomenon is
plant in other experiments,
tretamine and with cage
ivility for the 1st
as mated) [2], one
the vigour and male
ents the first fertilized
ivity, viability and hatch-
ent. In both treatments,
ind experiments with
amine [2]. Thus, the
age to the male
nsect numbers used
sion on mating activity
se. A preferential
and explain the lower
sterility induced in nympha
treatments; young adults have more sperm than
late-5th-instar male nymphs and the later more than the mid-5th
[2]. Fahmy and Fahmy [9] suggested that the mature spermatозоa are primarily
affected by tretamine and Jackson [10] found that the fully formed spermatозоa
and spermatids are far more sensitive to tretamine than earlier stages.

**Adult treatments**

Figures 1 - 3 present the whole reproductive system in control and
treated female bugs. As these pictures suggest, tretamine inhibits oocyte
development. Upon recovery (Fig. 3), some abnormally small oocytes
appeared in some of the ovarioles. On day 26 the control females had the
ovarioles full of growing oocytes and the lateral oviducts with many stored
ova; ovaries with no signs of recovery on adult day 26 presented a picture
similar to Fig. 2. Very few females were found recovering 5 days after
treatment. **Many chemicals have been used to induce female infecundity,**
which is usually attributed to severe damage to either the oogonia or
nutritive cells or both [11]. Hussein [12] showed that HMPA and metepa,
topically applied, decreased egg production by about 50% when *Lygus hesperus* females were treated. Morgan [13] found that female house flies maintained on food containing 1% or 2% heme showed inhibition of egg development followed by vacuolation in the nurse cells and oocytes. Smittle et al. [14] showed that German cockroach females injected with tepa produced few or no oothecae.

In previous work applications of tretamine at 20 mg/kg (1 μg/bug) delayed egg production in milkweed bug females for about 10 days; the subsequent egg production rate was low and many eggs were abnormally small [2]. The above applications were on adult days 1–2 when no visible oocytes are seen in the ovarioles. (A minor experiment, on the development of ovaries at 30°C, showed that the first macroscopic signs of oocyte development usually start 3–4 days after the adult moulting). Table IV shows that one out of three eggs produced by *O. fasciatus* females, treated with 20 mg/kg of tretamine on adult day 2, had a significantly reduced size. These small eggs gave an extremely low hatch and almost none of the minute nymphs ended up as adults; most of them died during the 1st instar. Eggs of a normal size, laid by treated females, gave lower hatch and more embryonic death than the controls. The majority of small eggs developed an embryo to some degree seen inside). The percent usually show a figure nymphal survival is that experimental period, housed bugs injected phosphorothrihide), started growing older to the extent that one on the other hand, the the end of the experin. The above effect is well indicated by the supply in nutrients. 1 normal milkweed bugs pointed out that the wility but seems to be Leverich [16] suggest Cochliomyia hominiv
FIG. 3: Ovaries from a 26-day-old D. fasciatus female treated with 20 ng/kg of tetramine on the first day of adult life. Partial recovery of oocyte development can be seen in some ovarioles ×8.

An embryo to some degree but no hatch finally took place (red embryo seen inside). The percentage of adults developed from control eggs (22%) is not considered a representative one since observations in our laboratory usually show a figure of about 30%. The most likely explanation of that low nymphal survival is that for 4–5 consecutive days, in the middle of the experimental period, the control eggs were kept in jars which previously housed bugs injected with large quantities of DEF (S, S', S''-tributyl phosphorothioate), an aliphatic inhibitor. As the control females started growing older their eggs were often observed to be smaller but not to the extent that one could list them as being \( \frac{1}{2} \) of a normal-size egg. On the other hand the small eggs from treated females were fewer toward the end of the experiment, probably the result of a progressive recovery.

The above effect on fecundity indicates injury to the trophocytes. This is well indicated by the striking reduction in egg size, a result of deficient supply in nutrients. Richards and Kolderie [4] showed that small eggs from normal milkweed bugs have also a reduced hatchability, and Richards [18] pointed out that the weight of eggs is not only correlated with their hatchability but seems to be also related to successful hatching. LaChance and Leverich [16] suggested that g-irradiation hampered oocyte growth in Cochliomyia hominivorax presumably because of damage to the nurse cells.
### TABLE IV. REPRODUCTION VALUES IN MILKWEED BUG FEMALES TREATED WITH 1 µG OF TRETAMINE ON ADULT DAY 2

Equal numbers of normal males were added 5 days after treatment. The values are totals for a period of 20 days after mixing the sexes.

<table>
<thead>
<tr>
<th>Insects</th>
<th>Eggs examined</th>
<th>% Nymphs hatched</th>
<th>% Adults produced</th>
<th>% Eggs that had embryo inside but did not hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>% having size 1/2 - 1/3 of normals</td>
<td>From eggs of normal size</td>
<td>From eggs of small size</td>
</tr>
<tr>
<td>Treated</td>
<td>542 a</td>
<td>36.5</td>
<td>64.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Control</td>
<td>721 b</td>
<td>0.0</td>
<td>73.5</td>
<td></td>
</tr>
</tbody>
</table>

---

* a All eggs laid were collected and examined.

* b Only random samples of 40-80 eggs were examined daily.
The same researchers [17], working with an alkylating agent, (2, 5-bis(1-aziridinyl)-3, 6-bis[2-methoxyethoxy-p-benzoquinone]) on the same insect suggested that chemical mutagens affect the endomitotic process in nurse cell chromosomes (which leads to the formation of polytene chromosomes) resulting in reduced insect fecundity.

ACKNOWLEDGMENTS

Many thanks are due to Dr. H. T. Gordon of the Department of Entomology, University of California, for help during this work as well as Dr. J. S. Bowman for supplying the chemosterilant. I also wish to thank Mrs. V. Trouposklados for assistance in preparing the manuscript.

REFERENCES

DISCUSSION

L. E. LaCHANCE: As tretamine treatment of the 4th instar did not prevent the production of mature sperm, could you tell us whether spermatids were present at the time of treatment.

A. P. ECONOMOPOULOS: No spermatids were found in testes from late 4th O. fasciatus males. The 4th-instar was found to be the period of spermatocyte accumulation, so that at the end of the instar the testis was packed with numerous layers of spermatocytes. At the very beginning of the 3rd instar a wave of differentiation starts which proceeds toward the apical region of the testis; by the time the 5th-instar nymph is ready to molt into adulthood, half of the testis is full of sperm. Gordon and I have established this.