THE CHEMOSTERILISATION OF INSECTS WITH SPECIAL

REFERENCE TO CERTAIN LEPIDOPTERA.

A thesis submitted by

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ABSTRACT

44 potential chemosterilants were evaluated by injection
application to the noctuid Diparopsis castanea. The most
effective was tris-(1-aziridinyl) phosphine oxide (tepa). Sterility
in male moths was permanent although the insects were sexually
competitive. Male Autographa gamma were similarly sterilised.
Both mating effectiveness and oviposition were reduced when
female Diparopsis were injected with sterilising doses. Tepa
also sterilised male Diparopsis when applied topically in
acetone, after overnight contact on treated surfaces or by
probing on aqueous solution. In all three instances the
sterilised moths were sexually competitive.

Male Diparopsis were attracted to virgin females confined
in a prototype bait-station; but on arrival did not often probe
until at least 3 days old. Unless probing occurred the moths
were not sterilised by momentary contact. To facilitate
chemosterilant penetration after momentary contact, certain
oils were applied to male moths, but all reduced mating
effectiveness. Results from population models suggested that
the advantage of sterilising attracted males instead of killing
them would be slight.

Tepa was degraded rapidly when applied to male Diparopsis
topically or by injection at temperatures prevailing under
field conditions in Central Africa.

Tepa treatment of female _Diparopsis_ caused a degeneration of young oocytes within 3 days and a shrinkage of mature eggs. No marked effects on the testes of _Diparopsis_ or _Autographa_ were noted after similar treatment. Some eggs of both _Diparopsis_ and _Autographa_ from tepa-treated male parents showed no signs of embryological development, others developed considerably but did not hatch. A reduction of alkaline phosphatase in _Diparopsis_ testes, together with agglutination of sperm from tepa-treated males stored in female spermathecae after mating, suggested that a reduction in sperm motility had occurred; no damage to the sperm ultra-structure was however observed. Carbaryl and certain s-triazine chemosterilants caused various mating aberrations in _Diparopsis_, possibly associated with observed increased levels of endogenous biogenic amines.

CONTENTS

GENERAL INTRODUCTION

The sterile male method of insect population control is based on the principle that the introduction of sexually sterile but otherwise sexually vigorous males into the natural population of an insect species will have a greater influence on reducing the biotic potential of the population than the elimination of the same number of individuals from the population. Two systems can be employed when using sterility to suppress insect populations; one utilizes the mass production, sterilization and release of sterile organisms, the other involves the direct sterilization of the natural population. Several methods are available for causing sterility in insects, including gamma - radiation, chemosterilants, cytoplasmic incompatability, hybrid sterility and high intensity photoflash discharges. Only chemosterilants will be considered in this thesis; they are by definition chemical compounds capable of reducing or destroying the reproductive capacity of an organism to which they are administered. The potentialities of chemosterilisation for the control of insect pests have been elaborated by Knipling (1955, 1959, 1960, 1962, 1963). Apart from their use in sterilising mass-reared insects, particularly as lepidopterous insects are extremely radio resistant (North & Holt, 1968) the natural population insect might be sterilised by direct application methods. For the latter case a dual effect operates in that

(a) the sterile insect cannot reproduce and (b) the sterile
insect remains in the environment and will compete for the
remaining fertile insects. Even greater advantages occur
when the coverage of the environment for what ever reason
is incomplete and when the numbers of insect are low; in
which case the sterilised insect will actively seek out its
victim.

In the present work the possibilities of sterilising
lepidopterous insects are considered. Two species of noctuid
moth were chosen for study, the red bollworm Diparopsis
castanea (Hmps.) and the Silver Y Autographa gamma L. Both
insects are of economic importance and the possibility exists
of developing practical control methods involving chemosterilant
techniques. Diparopsis castanea is an important pest of
cotton in Central and Southern Africa and since it spends most
of its larval life inside the cotton boll it is particularly
difficult to control by insecticides. Autographa gamma
is a migratory species, the larvae of which are serious pests
of sugar beet in epidemic years in this country. The larvae
also attack cabbage, flax, potatoes, beans and a whole range
of wild plants.

The thesis is presented in 5 parts. Part I describes
and classifies the various groups of chemosterilants and the
effectiveness of representative members of each group
against Diparopsis. The effect of treatment on the sexual
vigour of the moths is further evaluated for those chemicals

showing sufficient sterilising activity. In part II possible

practical control procedures involving the use of chemosterilants

are discussed and in particular, experiments designed to

test the potential use of an autosterilising bait-station

for control of _Diparopsis_. The persistence of chemosterilants

when applied to insects is described in part III. In part IV

the effect of chemosterilants on the reproductive organs, sperm

and embryogeny of the two species of moth are described.

In part V possible relationships between chemosterilant action

and endocrine misfunction are considered.

PART I.

The classification of insect chemosterilants and an

evaluation of the sterilising activity exhibited by

representative members from each group.

INTRODUCTION AND REVIEW OF LITERATURE

1. Biological alkylating agents.

One of the first groups of chemicals found to sterilise insects are known as biological alkylating agents. Biological alkylation implies the introduction of a hydrocarbon radical, often containing elements other than carbon and hydrogen, into a molecule under physiological conditions. The principal classes of alkylating agents are sulphonic acid esters (alkane sulphonates), Nitrogen mustards (2-chloroethylamines) and ethyleneimines (aziridines) (Ross, 1962).

Mustard gas (bis-(2-chloroethyl) sulphide) was developed in the First World War as an irritant or vesicant, while during the Second World War further research led to the development of the nitrogen-mustards. It was Auerbach & Robson (1947a, 1947b) who produced conclusive evidence that mustard gas could induce true mutations, a property previously thought to be associated only with radiation. Independantly however Rapoport (1947) had discovered the mutagenic action of diethyl sulphate, ethyleneimine and several other alkylating agents, while as early as 1898 Ehrlich recognised the unusual pharmacological properties of ethyleneimines and the simplest of the expoxides ethylene oxide. He noticed that they caused internal cell destruction in tissues where active division was occurring. By 1950 more than 265 papers had been

published reporting the effects of 240 different chemicals

on genetical material (Herskowitz, 1951). Research indicated

that in general biological action was greater when the

molecules contained more than one functional group.

Consideration of this conclusion together with other relevant

data led to the propounding of the "cross-linkage" hypothesis

(Goldacre, et al, 1949). The hypothesis proposed

that chromosome breaks might be produced by the linkage

together of say two chromatids as the result of treatment,

so that mechanical damage followed when they were pulled

apart during the process of cell-division. Although

no doubt such a hypothesis was an over. simplification of the

truth it led to the idea that industrial cross-linking agents

might also have similar properties and this in turn led back

to a study of the ethyleneimines (Hendry et al, 1951). Cross

linking agents are used in the textile industry for such purposes

as the strengthening of synthetic fibres and the flame proofing

of natural fibres. Thus Reeves et al (1957) have described

the use of the ethyleneimines tepa and thiotepa as flame

retardants for cotton fibre, while Buckley et al (1951) showed

that tepa retarded the growth of Sarcoma 180 in mice. Damage

to the internal organs was similar to that caused by nitrogen

mustards. Both tepa and thiotepa are now used as palliative

agents in certain neoplastic deseases and in containing

adenocarcinoma of the breast and ovary. Among alkylating agents

of the Sulphonic acid type Myleran or Busulphan
(1:4-di-(methanesulphonoxy)butane) has found wide chemical
application in the treatment of the chronic blood desease myeloid
leukemia (Ross, 1962). A further discussion on the possible
mode of action of these chemicals will be found in Part IV.

In the search for insect chemosterilants initiated by
the United States Department of Agriculture in 1958, it was
argued that interference with the fertility of an organism
at the cellular level, would be similar to interference with
the reproduction of cells in a tumour. This assumption proved
to be correct since virtually all the earliest insect
chemosterilants were either cancer chemotherapeutic agents and
closely related compounds. These compounds have been listed
by LaBrecque, (1961); Crystal, (1963); Fye, (1967); Fye et al, (1965);
C. Smith et al, (1964); Borkovec et al (1968a). They induce
dominant lethal mutations in the mature ova and sperm of
insects but can also lower the fecundity of the females and
cause aspermia or sperm inactivation in the males. The
effect which predominates depends largely on the age of the
insect and the stage of the reproductive cells at the time
of treatment (Morgan & LaBrecque, 1962; Crystal & LaChance, 1963;
Rai, 1964; LaChance & Leverich, 1968). In general however the
alkylating agents are most effective against male insects and their
activities summarised in reviews by Wiedhaas & McDuffie, (1963) Ascher
(1964, 1969) Bertram (1964) Campion (1965) Borkovec (1966)

Gruner (1966) Kilgore (1967) Shumakov (1967) White (1967)
LaBrecque & Smith (1968) Stüben (1969).

The sulphonic acid type of alkylating agent has not
been so extensively investigated perhaps partly because of
the limited solubilities of the group. However Crystal (1968)
determined the effectiveness of a number of such compounds
against the screw-worm fly Cochliomyia hominivorax (Coquerel)
and found that methanediol dimethane sulphonate applied
topically caused complete sterility while the insects remained
sexually competitive. Klassen et al (1968) also found
busulphan or myleran (1,4-butanediol dimethane sulphonate),
1,3 propanediol dimethane sulphonate, and chlorambucil
(4 (p- (bis (2-chloroethyl) amino-)butyric acid) effective
as sterilants when fed to male boll-weevils (Anthonomus grandis).
Shaw & Sanchez Riviello (1962) found that chlorambucil
sterilised the Mexican fruit fly (Anastrepha ludens) when
administered in the adult diet although it was also toxic.

2. Antimetabolites.

Goldsmith & Frank (1952) first showed that several folic
acid antagonists such as aminopterin and amethopterin
(methotrexate), both also widely used in the treatment of
leukemia and neoplastic diseases, caused sterility when fed
to female Drosophila. This was later confirmed in the housefly
by Mitlin (1956) Mitlin & Baroody (1958) Mitlin, Butt &

Shortino (1957) and Levinson & Bergmann (1959). A whole series of biologically active substances were later found capable of inhibiting ovarian development following their incorporation into the larval or adult diet. Such substances have been broadly classified as antimetabolites. These are defined as chemicals structurally related to biologically active substances, that when incorporated into the organism cause a metabolic process to slow down or stop (Borkovec, 1966).

Crystal (1964a) in describing the effect of several folic acid antagonists against the screw-worm fly concluded that these substances primarily affect maturing eggs rather than mature eggs. Perrin-Waldaner (1969) however found that aminopterin caused atrophy of the testes when applied to Drosophila diet, while Matolín (1969) showed that 6-azauridine specifically sterilised male houseflies. Morgan (1967) Řazábová (1968) Řezábová & Landa (1967) studied the effect of several antimetabolites on ovarian development in houseflies. 5-fluoro-orotic acid inhibited oocyte development, while vacuolation of the nurse-cells was evident 48 hours after treatment, and eventually complete degeneration occurred. Following treatment with 6-azauridine ovarian growth was again completely inhibited, while histological study showed a proliferation of the folicular epithelium of the egg chamber and subsequent degeneration of nuclei and cell-membranes. Masner & Mácha (1968) working with the lime-bug Pyrrochoris

apterus noted that 6-azauridine not only directly affected
ovarian growth but also prevented the synthesis of juvenile
hormone from the corpus allatum, which became reduced in size.
Under these conditions the fat-body failed to synthesise
protein and vitellogenesis was prevented. Gruner (1968)
showed that injecteddoses of apholate caused a reduction in
corpus allatum activity in the Dynastid beetle Phyllognathus
silenus.Hence alkylating agents can also disturb endocrine
function. Kilgore & Painter (1962) and Painter & Kilgore (1965)
also showed that several antimetabolites including 5-fluorouracil,
5-fluorodesoxyuridine and amethopterin inhibited ovarian
development in houseflies. Similarly Akov (1967) demonstrated
that amethopterin inhibited egg-development in the mosquito
Aedes aegypti when added to the larval medium. Cycloheximide,
a broad spectrum antibiotic has shown chemosterilant activity
against female houseflies (LaBrecque & Gouk, 1963), aphids
(Harries & Wiles, 1966) ·· Mexican fruit flies (Shaw &
Sanchez Riviello, 1962), and the eye-gnat Hippelates collusor, Mulla
(1968) although generally with toxic side effects. The antibiotic
Anthramycin methyl ether effectively sterilised Drosophila
Barnes et al (1969).

Another antibiotic Actinomycin D reduced oviposition
without a significant effect on mortality when applied to
the two spotted mite Tetranychus urticae (Koch) by Harries
(1968). Kenaga (1969) described the sterilising activity of
a series of hydroxy nitrosamino aliphatic acids related to

the antibiotic alanosine. When encorporated into the diet of houseflies some of these induced sterility in the female insects at a concentration of 250 ppm. A series of dinitro-napthalene benzene sulphonanilides were also active as chemosterilants against female houseflies.

3. Further azirdine derivatives.

After the effectiveness of tepa and related compounds as chemosterilants, against a number of insect species had been demonstrated, Borkovec & co-workers synthesised several series of related compounds to determine the effect of various substitutions on chemosterilising activity and to possibly develop a structure-activity relationship. Initially Woods, et al, (1964) : prepared a series of N-acylaziridines which were tested against houseflies Musca domestica, screw-worm flies Cochliomyia hominivorax and Mexican fruit flies Anastrpha ludens by feeding them a diet containing 1% of the candidate chemosterilant. 20 out of 25 were active as chemosterilants, although none were was effective as tepa or apholate. The basic formulae are shown in Fig. 1.
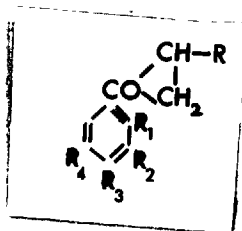
Fig. 1. aromatic acylaziridine   aliphatic acylaziridine
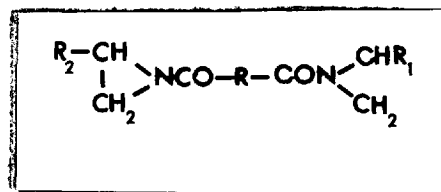
A similar series of aromatic derivatives were prepared by Geering et al (1965). In further experiments (Borkovec & Woods, 1965), the acyl groups were replaced by carbamoyl groups. Compared with similar acyl compounds, the monofunctional aziridines were only slightly active. A greatly enhanced chemosterilant activity was demonstrated in a difunctional series of carbamoyl compounds however. Thus 1,1'-suberoyl-bis-aziridine (Fig. 2,I) was inactive against houseflies, while the corresponding carbamoyl, N,N'-hexamethylene,bis,1-aziridine carboxamide eliminated eggs hatch at a concentration in the diet of 0.1% (Fig. 2,II)
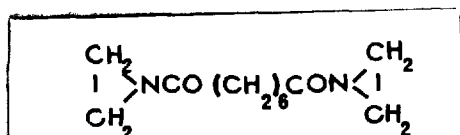
Fig. 2.　I　1,1'suberoyl bis-aziridine
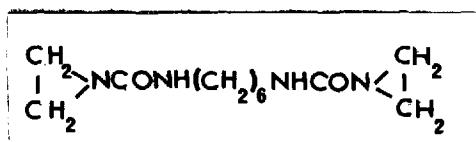
II　N,N' hexamethylene, bis-1-aziridinyl carboxamide

Replacement of an entire aziridinyl moiety by another group resulted in compounds of high sterilising activity (Borkovec, et al, 1966), . and of greater activity than

the present compound tepa.

A methylamino derivative, monomethyl bis (1-aziridinyl)
phosphine oxide (Fig. 3) is one of the most potent chemosterilants
known (Chang & Borkovec, 1966b).
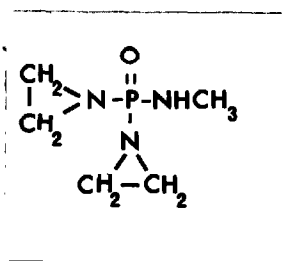
Fig. 3. Mono-methyl, bis (1-aziridinyl) phosphine oxide.

However, progressive replacement of aziridinyl groups in
tepa by dimethylamino groups led to a steady decrease in the
sterilising effect resulting in the trisubstituted dimethyl-
amino compound HMPA (hexamethyl phosphine triamide), which
however still retains some sterilising activity (Chang,
et al, 1964   ; LaBrecque et al, 1966).

A similar replacement of one aziridinyl moiety by methylene
groups also produced chemosterilants of high   activity
against houseflies. Vashkov et al, (1969)   synthesised a
series of chemosterilants based on thiotepa. "Demp" sterilised
only males while "Deep" was effective against both sexes.
Kpopachova et al (1969) found that bis (diethyleneimide)
N,N, piperizinyl amido phosphoric acid and bis (diethyleneimide)
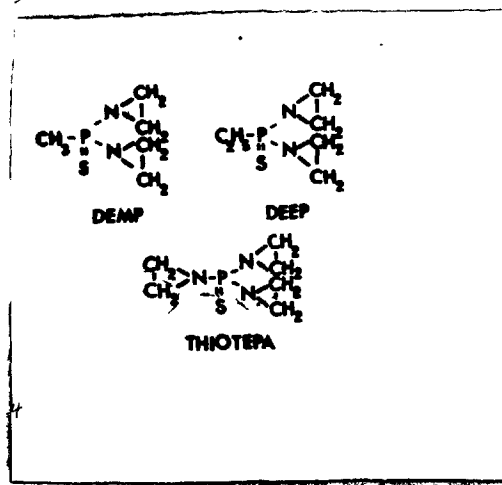
Fig. 4. Structural formulae of DEMP., DEEP and THIOTEPA.

6-morpholino-pyrimidyl-4-amidophosphoric acid were active as chemosterilants against a range of insect species and both were considerably less toxic to houseflies than thiotepa.
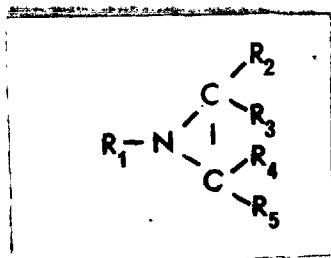
Fig. 5. Basic aziridine structure.

Borkovec, et al (1968a)   concluded from the results

of screening 301 aziridines against houseflies that sterilising

activity of an unsubstituted or C-substituted aziridine could

be increased by appropriate N substitution (R_1) but not by

C substitution (R_2 - R_5). Also polyfunctional compounds were

generally more active than monofunctional compounds which was

also noticed by Ristich et al (1965), who evaluated a series

of apholate analogues for chemosterilant activity also against

the housefly. It was also concluded by Borkovec

et al, (1968a) that the only types of substituents that

produced effective sterilants were those containing polar high

electron density systems like $C=O$, $P=O$, $S=O$, $C=N$ etc., while

compounds substituted by electron withdrawing substituents

were inactive.

4. Phosphoramides and s-triazines.

However interesting such variations in activity, the fact

remains that they are all aziridines, with the exception of HMPA

and present the similar disadvantage for use as insect control

agents in the field as tepa, in that they would expose man to

considerable mutagenic hazard. Mutagenicity has even been

shown for HMPA (Palmquist & LaChance, 1966) so that even such

apparently safer compounds should be approached with caution

In an effort to find a safer chemosterilant, a series of

phosphoramides related to HMPA was synthesised (Terry & Borkovec,

1967) but out of 50 compunds prepared, only hoxamethylthiophosphine
triamide sterilised houseflies as effectively as HMPA.
Replacement of one or more methyl groups in either HMPA or
thioHMPA with either higher alkyls or hydrogen, invariably led
to a decrease in activity. HMPA is inactive against boll-weevils
Anthonomus grandis (Klassen et al, 1968) and exhibits only
weak sterilising activity against other insects including
Mexican fruit fly Anastrepha ludens, the screw-worm Cochliomyia
hominovorax, the cucumber fly Austrodacus cucumis, the olive fly
Dacus oleae, the eye-gnat Hippelates collusor, azuki bean weevil
Callosobruchus chinensis and codling-moth Carpocapsa pomonella.
A survey of the chemosterilant activity of phosphoramides
applied to adult insects is presented in Table 20.

Following the discovery that hemel (tris,2,4,6- dimethyl-
amino-s-triazine) exhibited weak chemosterilant activity
against the housefly and a few other insect species (Chang,
et al, 1964, Borkovec & Terry, 1965), further possible
structural/activity relationships based on hemel as the parent
compound were studied by Borkovec & De Milo (1966). The
basic structure of the group is shown in Fig. 6 and is analogous
to the aziridine alkylating agent tretamine or TEM, a very
powerful mutagen.

One s-triazine compound, 2,4-diamino-6-(2-furyl)s-triazine
has shown high chemosterilant activity against houseflies,
but is inactive against mosquitoes, screw-worm flies and
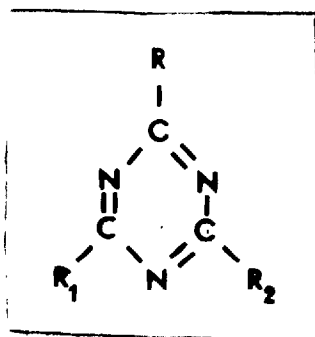
Fig. 6. Basic s-triazine structure.

Mexican fruit flies (Borkovec, 1966). Hemel itself, although
active against houseflies is only slightly active against
codling moth (Borkovec & Terry, 1965), and is inactive against
boll-weevils (Klassen et al, 1968).

In further examples of apparent species specificity,
bis-(dimethylamino)-piperidino phosphine oxide and
bis-(dimethylamino) morpholino phosphine oxide have actively
sterilised houseflies but were inactive against boll-weevils and
tobacco budworms (Borkovec, private communication; Klassen
et al, 1968; Flint et al, 1968a & 1968b). A series of s-triazines
of known herbicidal properties were completely inactive against
houseflies (Borkovec et al, 1967). LaBrecque et al (1968)
tested 110 variously substituted s-triazines for chemosterilant
action against houseflies. Several of those chemicals sterilised
male insects as effectively as hemel although they were closely
related in structure. It was generally concluded that the
inhibition of egg-hatch from treated flies was more affected
by polysubstituted compounds (3-4 substituents) than by less

substituted compounds. Compounds that were effective inhibitors
of pupation were uneffective as male sterilants. and these
were mono or di substituted compounds, (possible causes of such
activity are discussed in Part V). A survey of the varied
chemosterilant activity of s-triazines applied to adult
insects is presented in Table 3.

5. Organo-metals.

An entirely different group of chemicals of reported
chemosterilant activity is the organo-tin compounds. These
can be divided into four groups, having the general structure
$RSnX_3$, R_2SnX_2, R_3SnX and R_4Sn where R represents an alkyl
or acyl group and X is an inorganic radical not attached to
the tin by a tin-carbon bond. Workers investigating the
fungicidal activity of these compounds showed that the type
R_3SnX had much higher biological activity than the other three
groups (van der Kerk & Luitjen, 1954). Of these triphenyl
tin acetate and triphenyl tin hydroxide have been developed
commercially for use in controlling potato blight. The
molluscidal properties of certain triphenyl tin and also
triphenyl lead compounds have also been demonstrated and show
promise for the control of the vector of the debilitating
disease bilharzia (Hopf et al, 1968). The triphenyl metal
compounds have not shown marked insecticidal activity but
triphenyl tin acetate and triphenyl tin hydroxide showed anti-

feeding properties when tested against larvae of the Egyptian cotton leaf-worm Prodenia litura F. (Ascher & Nissim, 1965) and also against housefly larvae (Ascher & Moscowitz, 1968). Also chemosterilant activity has been reported for triphenyl tin acetate and triphenyl hydroxide when added to the diet of the adult housefly (Kenaga, 1965; Ascher & Nissim, 1964), while Nagasawa, Shinohara & Shiba (1967) reported the sterilising activity of triphenyl tin hydroxide against the azuki bean weevil Callosobruchus chinensis L.

However Ladd (1968) showed that for the Japanese beetle Popillia japonica Newman triphenyl tin chloride acetate and hydroxide only induced sterility at levels associated with considerable mortality. Hays (1968) using triphenyl tin acetate and triphenyl tin chloride against houseflies concluded that both compounds compared favourably with tepa in reducing hatch when offered to sexually mature females. Ascher, et al, (1968) studied the sterilising effect of triphenyl tin acetate in male houseflies and concluded that the main effect was achieved by a slow poisoning of the sperm manifested by a protracted immobilisation and kill of the sperm in the spermathecae of the females. However Pate & Hays (1968) found that triphenyl tin acetate and triphenyl tin chloride in rats, indeed produced degenerative changes in testicular tissue when administered at dosage rates of 10-40 mg/kg/day over a period of 19 days, while Newton & Hays (1968)

showed that these compounds also produced significant changes in the ovaries at similar concentrations.

Mulla (1968) tested triphenyl tin acetate, triphenyl tin chloride and triphenyl tin hydroxide by feeding them to adult eye-gnats _Hippelates collusor_ , but very little sterility was induced at concentrations in the diet of 0.1, 0.5 and 1% levels.

A series of compounds based on boron were investigated as chemosterilants against the screw-worm and houseflies (Borkovec _et al_, 1969; Settepani _et al_, 1969). When incorporated into the adult diet, several showed weak sterilising activity against female insects, thus showing characteristics of an antimetabolite. A series of 14 Vanadium compounds were investigated by Crystal (1970) as possible chemosterilants for the screw-worm fly. Oral treatment induced sterility in some instances but only at doses also causing high mortality. As in the case of the boron series, only female insects were predominantly sterilised.

6. Miscellaneous compounds.

Insect chemosterilant activity has also been reported from a wide range of apparently unrelated chemicals. Battacharya (1949) observed sterility in male _Drosophila_ reared on food containing ethylene glycol. The induction of sterility in both male and female houseflies has been reported following the incorporation into the insect diet of m-xylohydroquinone

(Ascher & Hirsch, 1963; Ascher & Avdat, 1967). Since Kar et al

(1963) failed to show any antifertility effects with this

compound even after prolonged administration to male rats,

this may be an example of an insect specific chamosterilant.

A number of urea and thiourea derivatives have been tested

(Borkovec, 1966). Thiourea for example exhibits a rather

erratic sterilising effect perhaps associated with its high

toxicity (Mitlin & Baroody, 1958; Gouk & LaBrecque, 1963

LaBrecque et al, 1960; Piquett & Keller, 1962). Hydroxyurea

caused morphological changes in the ovaries and egg-cells of

the housefly (Kissam et al, 1967). Another urea derivative

4-imidazolin-2-one is claimed to be a potent inhibitor of

reproduction in several species of insects and with low

toxicities to birds and mammals (Schaeffer & Tieman, 1967).

For example, newly emerged adult milkweed bugs Oncopeltus

fasciatus were sterilised by an injected dose of 2.5 µg.

Griseofulvin is an antifungal antibiotic widely used for

the therapeutic control of dermatophytes. It causes hyphal

distortion by interfering with chitin synthesis. Preliminary

experiments with Drosophila (Campion unpublished data) had

suggested that the antibiotic had chemosterilant activity,

while Malawista,   et al,   (1968) demonstrated that

griseofulvin reversibly disrupted the living mitotic spindle

in the marine annelid Pectinaria gouldi.

Sterculic acid belongs to an unusual class of fatty acids found in certain plant lipids, and contain the cyclopropane ring (Fig. 7).

$$R_1-C = C-R_2-COOH$$
with CH_2 bridging the two carbons
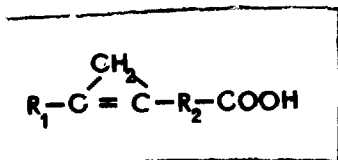
Fig. 7. Basic structure of sterculic and malvalic acids.

When oil from Sterculia foetida, containing from between 44-77% sterculic acid was incorporated into the diet of adult houseflies at a concentration of 2.5-5.0%, the female flies were completely sterilised, while partial sterility occurred in the males (Beroza & LaBrecque, 1967). The tranquillising drug reserpine and the vitamin biotin both inhibited oviposition in the housefly when added to the insect diet, (Benschotter, 1966, 1967; Hays & Amerson, 1967; Wicht & Hays, 1967).

The antimicrobial agent sorbic acid suppressed larval development after treatment of the adult Dermestid stored product pests Attagenus megatoma and Trogoderma parabile (Boush et al, 1968).

7. Insecticides.

Many reports exist on the effect of sublethal doses of insecticides in reducing fecundities in insects; reviewed up to 1955 by Knutson and later by Moriarty (1969). To take just a few examples, Tattersfield & Kerridge (1955) showed that DDT reduced oviposition in Drosophila, on the other hand low doses

of aldrin and BHC stimulate oviposition in the pink bollworm
(Adkinson & Wellso, 1962; Williams et al 1958). Working with
houseflies, Georghiou (1965) showed that although mating
ability and subsequent egg fertility were unaffected by
sublethal doses of isolan and related carbamates including
carbaryl, a significant decrease in fecundity occurred whether
applied before or after mating. The sterilising effects of
a series of carbamates against Drosophila were noted by
Rapoport (1962). Other Russian workers found that DDT caused
pathological changes in the ovaries of the Musca domestica and
Protophormia terraenovae (Derbeneva-Uhova

et al, 1966).while conversly Sutherland et al,
(1967) found that exposure of Aedes aegypti larvae to DDT
stimulated a 34% increase in the production of ovarioles with
follicles. Ramade (1967) and Kuipers (1962) have both
suggested that the neurosecretory system may be affected as
the result of treatment. These possibilities will be considered
in greater detail in Part V.

8. Juvenile hormone analogues.

A number of insect hormone mimics related chemically
to farnesol and which almost certainly cause neurosecretory
disturbances may have application as insect chemosterilants.
Masner et al, (1968) and Matolín (1970) showed that
female lime bugs Pyrrhochoris apterus were rendered sterile by

injection of 1 µg amounts of methyl farnesoate dihydrochloride
(DMF), although they could well tolerate a dose 10,000 times
the sterilising dose. Males treated with 1000 µg of DMF
transmitted 5-7 µg of the substance to the female bug during
copulation and in so doing caused female sterility. Repeated
matings by the treated males with either virgin or previous.y
mated female insects had the same effect. The substance was
however apparently specific for Pyrrhochoris. Only slight
sterilising effects were obtained when houseflies were
treated at high levels of application, and no activity at all
against the Lepidoptera Pieris brassicae and Galleria
melonella (Sláma private communication). However Riddiford &
Williams (1967) sterilised silkworm females by injection of
DMF dissolved in ceoropia oil. They also found that DMF
applied topically to silkworm eggs prevented hatch at a dose
0.4 µg per egg if applied during the first 5 hours after
oviposition but after this time the egg was resistent.

The insect moulting hormones, the ecdysones and their
analogues are also capable of inhibiting insect reproductive
processes. Robbins et al (1968) showed that natural 20-
hydroxyecdysone, ponasterone and a synthetic ecdysone
analogue inhibited ovarian maturation and egg production when
injected into adult houseflies, 20-hydroxyecdyson similarly
inhibited ovarian maturation in the stable-fly Stomoxys
calcitrans when added to citrated bovine blood at a concentration
of 1.0% and fed to 1 day old insects for 5 days (Wright &

Kaplanis, 1970).

Having considered chemosterilants and their activities
in general, a closer examination of their action on Lepidopterous
insects in particular is now described.

Sterilisation of Lepidopterous insects.

Treatment of the egg.

Newly deposited eggs of the gypsy moth Porthetria dispar
were treated with 0.05 - 5.0% solutions of the alkylating
agents tepa, metepa and apholate for periods of 1-30 minutes.
The chemosterilants were dissolved in water containing the
wetting agents Tween 20 or Tween 80 at concentrations of 0.5%.
Subsequent examination of the eggs showed that no reduction
in hatch occurred while adult moths were of normal fertility
(Collier & Downey, 1965). High egg mortality occurred when
eggs of codling moth Carpocapsa pomonella at the age of 4 days
were immersed for one minute in 5% tepa dissolved in acetone,
(Hathaway et al, 1966).

Treatment of larvae (a) incorporation
into the diet.

Treatments of larvae were made by either incorporating
the chemosterilant into the larval diet at varied concentrations
or topically applying known amounts of chemosterilant.
Hensley & Mathern (unpublished data) for example incorporated

tepa into an artificial diet for sugar cane borer larvae Diatraea saccharalis, at a concentration of 0.5%. This resulted in high larval mortality, although only a slight reduction in fecundity and fertility of the surviving adults occurred. Similarly when apholate was mixed with flour and fed to 1st and 3rd instar Mediterranean flour moth larvae Ephestria kuhniella; although the results were subject to considerable variation, it was concluded that at a concentration in the diet of 1%, complete sterility in subsequent adults was achieved although this was associated with high mortality and lack of competitiveness (Pelerents & Degheele, 1967).

Similar results were obtained by Harding (1967), when tepa and apholate were incorporated into the larval diet of the European corn borer Ostrinea nubilalis; in that either excessive larval mortality occurred or the resulting adults were not sexually vigorous. Sugai & Hirano (1965) and Sugai (1967) reported the sterilising effects of apholate against Bombyx mori L. when administered in the larval diet, although no tests on the vigour of the emerging adults were made. When hempa was incorporated into the larval diet of the gypsy moth Porthetria dispar at the 1% level very few larvae survived pupation to produce adults of normal appearance (Downey, 1968).

Treatment of larvae (b) topical

application.

Hathaway et al (1966) treated mature larvae of the codling

moth Carpocapsa pomonella by topical application of 15-80 μg

of tepa dissolved in acetone; Toppozada et al (1966) topically

treated 4th instar larvae of the Egyptian cotton leaf worm

Prodenia litura with tepa, metepa and apholate. It was

concluded that treatment of larvae caused excessive mortality

or other deleterious side-effects at doses necessary to cause

complete sterility in the few insects surviving to the adult

stage.

Treatment of the pupa

Pupae have been treated either by immersion in chemosterilant

solutions by injection or topical application.

Gypsy moth pupae Porthetria dispar at the ages of 0 hours,

24 hours and 5 days were immersed for periods of 10-30 minutes

in aqueous solutions containing 0.5% tepa, metepa or apholate

plus 0.5% wetting agent Tween 20 or Tween 90 (Collier &

Downey, 1965). High mortality of pupae aged 0-24 hours

occurred, although the reproductive capacity of survivors

was unaffected. Treatment of older pupae caused neither

mortality or subsequent adult sterility. Further experiments

(Collier & Downey, 1967) showed that when 0-12 hour pupae

were exposed for 2 minutes to 0.05% tepa, then 50% mortality

occurred while exposure for 30 minutes resulted in 92%
mortality. As before when older pupae were treated no
mortality effects were noted. When mature pupae of the
codling moth Carpocapsa pomonella were immersed in a solution
of 5% tepa dissolved in acetone for 1 minute, partial sterility
was induced. Using 10% tepa solutions, pupal mortality was
52% while an egg-hatch of 17% resulted from mating of the
survivors (Hathaway et al, 1966). Pupae of the fall army worm
Spodoptera frugiperda, less than 24 or 48 hours old were dipped
in aqueous solutions of apholate for 2 hours at concentrations
of 0.5-4.0%. Other pupae were treated with tepa dissolved in
10% aqueous solutions at concentrations of 0.1-10% by rotating
them in the solutions for 4 hours. No reduction in fertility
was achieved with any of the treatments (Young & Cox, 1965).
Similarly Pelerents & Degheele (1967) showed that pupal
immersion of Ephestia kuhniella in aqueous solutions of tepa
induced only partial sterility in the resulting adults.

Wellington & Maelzer (1967) found that female pupae of
the western tent caterpillar Malacosoma pluviale treated
topically with the large doses of the juvenile hormone
analogue farnesyl methyl ether, subsequently produced as adults
eggs that failed to hatch. Similar observations although of a
more erratic nature were noted by Outram. I. (private
communication) after topical application of farnesol,
farnesenic acid and farnesyl mether, to pupae of the spruce
bud-worm Choristoneura pluviale.

Treatment of Adults.

Considerable attention has been paid to the possibilities of sterilising the adult phase and the results broadly summarised in Table 1.

Oral uptake of azridine chemosterilants, generally in conjunction with sugar solution caused sterility in the Mallow moth Pectinophora malvella and the turnip moth Agrotis segetum (Shumakov et al 1966; Azarian et al, 1968; Bulyginskaya & Gruzova, 1968) the dark sword grass moth Agrotis segetum (Azarian et al, 1968, the American bollworm Heliothis zea; the tobacco budworm Heliothis virescens (Soto & Graves, 1967; Flint et al, 1968b), the cabbage moth Mamestria brassicae (Bonnemaison, 1966), the Egyptian cotton leafworm Prodenia litura (Toppozada et al, 1966), the armyworm Pseudaletia separata (Chang J. et al.1963), the beet armyworm Spodoptera exigua (Shumakov et al, 1966; Bulyginskaya & Gruzova, 1968) and the fall armyworm Spodoptera frugiperda (Young & Cox, 1965), the cabbage looper moth Trichoplusia Ni (Howland et al, 1965), the diamond back moth Plutella maculipennis (Bonnemaison, 1966; Hooper, 1969) and the codling moth Carpocapsa pomonella (Shumakov et al, 1966;Bulyginskaya & Gruzova, 1968). Oral uptake of aziridine chemosterilants was reported ineffective for the rice stem borer moth Chilo suppressalis (Pathak, 1968).

Topical application of aziridine chemosterilants dissolved in acetone sterilised the Carpenter worm moth Prionoxystus

Table 1. Chemosterilisation of adult Lepidoptera. A survey of the activities of chemosterilants and methods of application.

Family	Species names	Common name	Chemosterilant	Sex treated	Method of application	Activity [a]	Reference
Bombycidae	Bombyx mori	Silkworm	apholate	both sexes	oral	++	Hirano (1965)
Cossidae	Prionoxystus robiniae	Carpenter moth	tepa	males	topical	++	Solomon (1966)
Crambidae	Chilo suppressalis	Rice stem borer	apholate, tepa tretamine, hempa	both sexes	oral	+	Pathak (1968)
Gelechiidae	Pectinophora gossypiella	Pink bollworm	metepa	males females	topical, contact topical	++ +	Ouye et al (1965) Ouye et al (1969)
	Pectinophora malvella	Mallow moth	apholate, thiotepa, tretamine	both sexes	oral	++	Azarian et al (1968) Bulyginskaya (1965) Bulyginskaya et al (1968) Shumakov et al (1966)
Lymantriidae	Porthetria dispar	Gypsy moth	apholate, metepa, tepa	both sexes	contact	++	Collier & Downey (1967)
Noctuidae	Agrotis segetum	Turnip moth	apholate, thiotepa, tretamine	both sexes	oral	++	Azarian et al (1968) Bulyginskaya (1965) Bulyginskaya et al (1968) Shumakov et al (1966)
	Agrotis ypsilon	Dark sword grass moth	apholate, thiotepa, tretamine	both sexes	oral	++	Azarian et al (1968) Bulyginskaya (1965)
	Heliothis virescens	Tobacco budworm	apholate, metepa, & other aziridines	both sexes	oral	++	Soto & Graves (1967) Flint et al (1968a) Flint et al (1968b)
	Heliothis zea	American bollworm	apholate	both sexes	oral	++	Soto & Graves (1967)
	Mamestra brassicae	Cabbage moth	tepa, triphenyl tin acetate	both sexes both sexes	contact oral	++ -	Bonnemaison (1966)
	Prodenia litura	Egyptian cotton leafworm	apholate, metepa, tepa	both sexes	oral	++	Topposada et al (1966)
	Pseudaletia separata	Armyworm	thiotepa	both sexes	oral	++	Chang J. et al (1965)
	Spodoptera exigua	Beet armyworm	apholate, thiotepa, tretamine	both sexes	oral	++	Bulyginskaya et al (1968) Shumakov et al (1960)
	Spodoptera frugiperda	Fall armyworm	apholate, tepa	both sexes	oral	++	Young & Cox (1965, 1968)
	Trichoplusia ni	Cabbage looper	apholate, tepa	both sexes	oral spray	++ ++	Howland et al (1965) Henneberry & Kishaba (1966)
Phycitidae	Cadra (=Ephestia) cautella	Flour moth	apholate	both sexes	oral	+	Gangrade & Pant (1970)
Plutellidae	Plutella maculipennis	Diamond back moth	tepa, metepa, hempa, triphenyl tin acetate	both sexes both sexes both sexes	oral & contact oral oral	++ + -	Bonnemaison (1966) Hooper (1969)
Tortricidae	Carpocapsa pomonella	Codling moth	tepa, apholate, thiotepa, tretamine antibiotics	both sexes both sexes females	topical oral topical	++ ++ -	Hathaway et al (1966) Azarian et al (1968) Harris (1967)

a, ++ = active, + = slightly active - = inactive

Solomon (1966)

robiniae, the pink bollworm Pectinophora gossypiella (Ouye
et al, 1965, 1969), the tobacco budworm Heliothis virescens
(Flint et al, 1968a), the diamond back moth Plutella maculipennis
(Hooper, 1969) and the codling moth Carpocapsa pomonella
(Hathaway et al, 1966). Induction of sterility by contact with
surface films of chemosterilants was shown in the pink bollworm
(Ouye et al, 1965), the gypsy moth Porthetria dispar (Collier
& Downey, 1967), the cabbage moth Mamestria brassicae
(Bonnemaison, 1969) and the diamond back moth Plutella
maculipennis (Hooper, 1969).

The few other potential chemosterilants tested including
hempa, triphenyl tin acetate and various antibiotics were
uneffective (Bonnemaison, 1966; Harries, 1967, Pathak, 1968).

Competitive mating tests.

Sterilised insects must be sexually vigorous enough to
compete with the untreated insects in the field. Competitive
mating tests are designed to assess this ability. Few rigorous
tests have been carried out using lepidopterous insects.
Male pink bollworms Pectinophora gossypiella sterilised by
topical application of Metepa at a dose of 15µg were fully
competitive in the laboratory (Ouye et al, 1965). Reduction
of the wild population enclosed togther with cotton plants in
a cage (6 x 6 x 36 ft) was achieved by an estimated seasonal
release of 7:1 sterile males to normal males (Ouye & Graham, 1967).

Competitive mating tests for the cabbage looper _Trichoplusia ni_ were also arranged in outdoor cages (10 x 24 x 6 ft), set out over 4 rows of cabbage plants. The newly emerged males were sterilised by feeding them on 1% tepa contained in 10% sugar solution or exposed for 2 hours on tepa treated glass. They were released in the field cages with sterile male: untreated male: untreated female ratios of 20:1:1, 15:1:1, 10:1:1 and 5:1:1 and 0:1:1. The number of larvae on 72 to 150 plants selected at random were counted 18 days after the moths were released. When treated by feeding, 73% control occurred at a release ratio of 10:1:1 while no reduction at all occurred at 5:1:1. Similar results were obtained when males were sterilised by contact with tepa treated glass surfaces; while at the highest ratio tested of 20:1:1 the larval population was reduced by 97% (Howland et al, 1966).

A different method was used for assessing the competitiveness of sterilised gypsy moths _Porthetria dispar_. Three wooded 1-acre plots were isolated from other wooded land by clearways. In each plot 130 virgin females were placed in one hundred 1-way baffle traps which would allow males to enter but not to escape. The traps containing the females were arrayed in 3 concentric rectangles within the plot. With the traps in place 50 sterile and 50 untreated males were released, the sterile males being marked on their wings with dye. After 2 days the eggs and females were collected from the

traps and the eggs collected assessed for hatch. The results indicated that the sterilised males could compete in a natural situation (Collier & Downey, 1967).

It is concluded from this survey of the chemosterilisation of lepidoptera that treatment of egg, larva or pupa is unlikely to be effective. This is because either the chemosterilant has failed to penetrate the cuticle, it decomposes too rapidly, it causes excessive mortality or it results in deformed or sexually incompetant insects. The juvenile hormone analogues may prove to be an exception to these generalities, but this speculation will require further experimentation (see Part V).

Treatment of adult moths on the other hand with several aziridine chemosterilants and by a variety of application methods, often induced sterility. In the few cases where secondary tests have been carried out, the sterilised insects were sexually competitive. Apart from the aziridine alkylating agents very little information exists on the relative activity of the other classes of chemosterilants referred to in the main introduction, against a lepidopterous insect.

The aim of the first series of experiments in the present study was to provide such a primary evaluation using adult Diparopsis. The selection of the most suitable chemosterilant available, for eventual field use was then achieved by means of a graded series of laboratory tests. Since from a theoretical point of view it is generally assumed that the male insect is

the most important target in both radio- and chemosterilisation, most emphasis in the present study is given to the effect of chemosterilants on the male moth. However it is noted that Husseiny & Madsen (1964) in their study of the sterilising effect of radiation on the Navel orange worm _Paramyelosis transitella_ concluded that the use of sterile females could be used as successfully as sterile males.

Milam & Galun (1967) from theoretical considerations deduced that the introduction of sterile individuals of both sexes for control of population was never inferior and sometimes even superior to the introduction of one sex alone. Indeed the release of both sexes of sterile insects was used for the successful eradication of the screw-worm _Cochliomyia hominivorax_ (Coquerel) from the South-eastern United States (Knipling, 1958). and the melon fly _Dacus cucurbitae_ Coquillett. from the island of Rota (Steiner _et al,_ 1965). For this reason the possibilities of sterilising female insects were also considered.

MATERIALS AND METHODS

1. The experimental insects.

Adult Diparopsis moths were obtained from pupae
collected in the field in Central Africa and shipped to
England. The pupae were maintained under constant light at
25-27°C and uncontrolled humidity. Daily collections of newly
emerged moths were made and those used in experiments were
therefore of known age. They were completely immobile in
the light and constant illumination therefore prevented them
from premature mating.

Autographa gamma were reared at the Silwood Park
insectary on cabbage plants. Numbers were limited because of
disease problems. Newly formed pupae were collected daily,
sexed and confined individually in 2" x 1" glass specimen
tubes, and stored under continuous light condition at 27°C
and 70% R.H. until they emerged.

2. Methods of application and standard mating procedure.

In the primary evaluation tests one day old Diparopsis
of specified sex were treated either topically or by injection.
Dissolved in distilled water or acetone microlitre quantities
were injected into the haemocoel of the insect using a
Burkhard microdrop applicator fitted with a 1 ml Agla all

glass syringe and a 20G needle. Injections were made through
the dorsal abdominal wall near the junction of thorax and
abdomen. Topical treatments were applied to the ventral
abdominal surface. Just before treatment the insects were
anaesthetised by minimal exposure to carbon dioxide. After
treatment they were enclosed with one day old virgin insects
of the opposite sex, with two males and one female in each
mating container. The containers were disposable plastic
cartons ($3\frac{3}{4}$ x $2\frac{3}{4}$ in) which were lined with blotting paper on
which eggs were laid. Disposable petri dish covers were used
as lids. The cups were kept at 27°C and 70% R.H. with a
12 hour photoperiod under artificial lights for 6 days.
Neither water or sugar solution was provided throughout the
test period. Daily collections of eggs were made from each
cup and each batch incubated separately for 6 days at 27°C
and 70% R.H. Daily assessment of mortality was also noted.
Not less than 20 replicates were used for the assessment of
the effects of any one dose level of the candidate chemosterilant.
Where possible three dose levels were tested although this was
sometimes restricted because of either limited solubility or excessive
toxicity. A compound of limited solubility was tested at the
maximum dose possible and rejected if inactive at that dose.
Similarly a chemical found to be toxic at a level causing
insignificant sterility was not tested at a higher level.

The same mating procedure was used for tests with

Autographa. Unlike Diparopsis they were much more active during the day and needed to feed. Each mating container was therefore provided with a cotton wool pad saturated with a 5% aqueous glucose solution, on which the moths were observed to actively probe. Because of the limited numbers of the insects available, only one male and one female were placed in each mating container.

At the end of the test period, the female moths were dissected for the presence of spermatophores as proof of mating. The frequency of spermatophore transmission also gave some indication of whether the chemosterilant treatment was having a deleterious effect on mating performance. Only one spermatophore was usually found in the bursa copulatrix of Diparopsis females, whereas up to six were noted for Autographa. Eggs laid by unmated females were discarded.

Since the chemicals used in many instances had either known or suspected mutagenic properties, strict precautions were taken both in preparing the test solutions and in their application. Rubber gloves were worn for all operations and treatments carried out in a well ventilated fume cupboard. Soiled glassware was soaked overnight in weak acid solution before being washed, while disposable materials were incinerated. The chemicals were stored either in a refrigerator or a deep freeze.

3. <u>Estimation of the median sterilising and lethal doses of active chemosterilants.</u>

(a) <u>Sterilising effects.</u>

To gain quantitative information on the sterilising and toxic effect of these chemosterilants showing appreciable sterilising activity against <u>Diparopsis</u>, groups of moth of specified sex were treated with graded concentrations of the chemosterilant applied topically or by injection in the manner previously described, and the sterility effects recorded in the usual manner.

(b) <u>Mortality tests.</u>

After similar treatment of one day old moths, they were held in groups of 25-30 insects in plastic sandwich boxes (11 x 6 x $3\frac{1}{2}$ in) for a period of 4 days under the same conditions as the insects tested for sterility.

Dosage mortality and dosage sterility data were analysed by means of probit analysis (Finney, 1952). The significance of the reduction in oviposition as a result of treatment was assessed by the Mann-Whitney U test.

4. <u>Competitive mating tests.</u>

Treated males were released into cages (72 x 72 x 72 in) together with virgin females and untreated males in varying ratios. After one mating night the female moths were individually confined in glass specimen tubes lined with blotting paper. At least 10 eggs were oviposited before those

from mated females were assessed for fertility in the usual
way. In later tests the standard disposable cartons were used.
In each carton were placed one virgin female, one treated and
one untreated male. In each replicate test 39 to 99 replicates
were treated.

5. Repetitive mating tests.

In the repetitive mating tests each treated male Diparopsis
was confined individually to a mating container, into which
was introduced on successive nights a fresh one day old virgin
female. The females were removed each morning to glass
specimen subes (2 x 1 in) lined with blotting paper and the
eggs laid were assessed in the standard manner. The females
were eventually examined for spermatophores as proof of mating.
In each replicate test 33-39 insects were treated.

RESULTS

(A) Primary Evaluation.

1. <u>The effect of potential chemosterilants</u>

   <u>against male Diparopsis.</u>

The effect of potential chemosterilants against male

Diparopsis, with the data corrected for parallel controls

are summarised in Tables 2-5. No statistical significance

was attributed to small difference in sterility induced by

the various compounds. The criteria of a potentially useful

chemosterilant are the inducement of complete sterility with

no or little adverse effect of longevity or mating performance,

preferably at a low dosage level. It is clear that only the

aziridines tepa, apholate and metepa come into this category

(Table 2) with complete sterility occurring at doses of 2, 5

and 20 μg respectively and with no apparent adverse effects

on mating. The methane-sulphonate busulphan was inactive but

because of poor solubility only a dose of 2 μg was applied.

Of the five phosphoramides tested, hempa caused slight

sterility but only at a dose level of 100 μg when a considerable

increase in mortality and a reduction in mating occurred.

A similar effect was noted following application of thiohempa

(Table 3). Of the four s-triazines tested, hemel was the most

effective. 80% sterility was induced at a dose of 40 μg

Table 2. The sterilising effect of 4 biological alkylating agents against male _Dipcropsis._

Chemosterilant	Dose (μg)	% 4-5 day mortality *	Mating percentage of control mating	% sterility *
Tris(1-aziridinyl)phosphine oxide (tepa)	2	0	108	100
2,2,4,4,6,6-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine (apholate)	5	0	131	100
Tris(2-methyl-1-aziridinyl)phosphine oxide (metepa)	20	2	105	100
1,4-dimethane sulphonyloxybutane (busulphan)	2	0	45	0

* Adjusted for control mortality and sterility by Abbot's formula.

Table 3. The sterilising effect of 5 phosphoramides and 4 s-triazines against male
Diparopsis applied by injection.

Chemosterilant	Dose (µg)	% 4-5 day mortality *	Mating as percentage of control mating	% sterility *
Hexamethyl phosphoric triamide (hempa)	100	40	53	37
Hexamethyl phosphoroioic triamide (thiohempa)	100	45	13	0
Bis(dimethylamino)piperidino phosphine oxide	100	80	120	1
Bis(dimethylamino)morpholino phosphine oxide	100	41	38	0
Hexamethylol phosphoric triamide (hydroxyhempa)	25	75	150	0
Tris-2,4,6-dimethylamino-s-triazine (hemel)	40	0	60	80
N^2N^2, N^4N^4, N^6N^6 hexamethylol melamine (hydroxyhemel)	30	67	100	49
N^2, N^4, N^6 trimethylol-melamine (trimethylol melamine)	4	4	46	22
2,4-diamino-6-morpholino-s-triazine hydrochloride	80	60	40	17

* Adjusted for control mortality and sterility by Abbot's formula.

Table 4. The sterilising effect of 5 organo-metal compounds against male Diparopsis applied
by injection.

Chemosterilant	Dose (μg)	% 4-5 day mortality *	Mating as percentage of control mating	% sterility *
Triphenyl tin acetate	10	0	37	30
Triphenyl tin hydroxide	15	0	105	50
Triphenyl lead acetate	10	0	100	0
Triphenyl lead hydroxide	1	0	34	0
Triphenyl lead sulphide	5	100	44	12

* Adjusted for control mortality and sterility by Abbot's formula.

Table 5. The sterilising effect of 3 antibiotics, a thiourea derivative and an insecticide against male _Diparopsis._

Chemosterilant	Dose (µg)	% 4-5 day mortality *	Mating as percentage of control mating	% sterility *
7-chloro-4,6-dimethoxycoumaran-3-one-2-spiro-1'-(2'-methoxy-6'-methylcyclohex-2'en-4'-one) (griseofulvin)	20	38	83	32
Sterculic acid (40%)	1 µl	79	30	53
M-xylohydroquinone	5	55	44	8
4-imidazolin-2-one	4	65	60	16
1-naphthyl n-methyl carbamate (carbaryl)	0.1	39	152	0

* Adjusted for control mortality and sterility by Abbot's formula.

without adverse effects on mating or mortality (Table 3).
Slight sterilising activity was obtained by the organo-metal
compounds triphenyl tin acetate and triphenyl tin hydroxide
while the lead analogues were completely inactive (Table 4).
Poor solubilities prevented tests at higher dosage levels.
In the miscellaneous group (Table 5), oil containing sterculic
acid caused 53% sterility when applied at a dose of 1 µl, although
this was accompanied by a marked increase in mortality and
a reduction in mating.

Little or no chemosterilant activity was shown by
m-xylohydroquinone, 4-imidazoline-2-one and griseofulvin.
The insecticide carbaryl at the sub-lethal dose of 0.1 µg,
markedly stimulated mating activity although no sterilising
action was noted (Table 5).

2. The effect of potential chemosterilants against female Diparopsis.

The results concerning the sterilising effectiveness of
potential chemosterilants against female Diparopsis are shown
in Tables 6, 7 and 8. Tepa induced complete sterility at a
dose of 20 µg while oviposition was reduced (Table 6).
All the antimetabolites reduced oviposition. Cyclohexamide
caused complete sterility at a dose of 0.1 µg but was also
highly toxic, griseofulvin induced 41% sterility at a dose of
30 µg with only slight mortality and no reduction in mating

effectiveness. 5-fluorouracil induced 21% sterility at a dose
associated with a considerable reduction in mating.
Reserpine caused both a reduction in egg-hatch and oviposition
with minimal toxic side-effects (Table 7). The phosphoramide
hempa caused 59% sterility only at the massive dose of 250 µg,
while thiohempa was virtually inactive at a dose of 100 µg.
Only very weak sterilising activity was noted for hemel and
the two other s-triazines tested (Table 6).

An increase in the oviposition rate was noted following
treatment with 80 µg of 2,4-diamino-6-morpholino-s-triazine HCl
and 0.2 µg of carbaryl (Table 7). Sterilising activity was
shown by the juvenile hormone analogues DMF, farnesyl
diethylamine and farnesyl methyl ether only at doses of 500 µg
and 1000 µg. Farnesyl methyl ether was the most active
inducing 75% sterility at a dose of 500 µg; although
considerable mortality and reduction in mating also occurred
(Table 8). The possibility of an alteration in the rate of
oviposition as the result of treatment, was assessed for each
mated female on subsequent days following treatment. The
results are presented in Table 9. Treatment with farnesyl
methyl ether at an injected dose of 500 µg caused an immediate
reduction in oviposition. Treatment with ethyl farnesoate
had no effect on the rate of oviposition for the first 2 days,
although on later days the number of eggs laid was consistently
fewer than those laid by untreated insects.

Table 6. The sterilising effect of an aziridine alkylating agent, 2 phosphoramides and 3 s-triazines against female <u>Diparopsis</u> applied by injection.

Chemosterilant	Dose (µg)	% 6 day mortality *	Mating as percentage of control mating	Oviposition as percentage of control oviposition	% sterility *
Tris(1-aziridinyl) phosphineoxide (tepa)	20	30	83	46	100
Hexamethyl phosphoric triamide (hempa)	250	10	70	81	59
Hexamethyl phosphoroioic triamide (thiohempa)	100	0	27	68	12
Tris-2,4,6-dimethylamino-s-triazine (hemel)	100	20	50	124	3
2,4-diamino-6-(2-furyl)-s-triazine	1	14	65	105	34
2,4-diamino-6-morpholino-s-triazine hydrochloride	80	0	122	122	26

* Adjusted for control mortality and sterility by Abbot's formula.

Table 7. The sterilising effect of 3 antimetabolites, a tranquillising agent, a teratogenic agent and an insecticide against female _Diparopsis_ applied by injection.

Chemosterilant	Dose (µg)	% 6 day mortality*	Mating as percentage of control mating	Oviposition as percentage of control oviposition	% sterility*
5-fluorouracil	30	60	33	42	21
B-(2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl)-2-glutarimide (cycloheximide)	0.1	100	33	22	100
7-chloro-4,6-dimethoxycoumaran-3-one-2-spiro-1'-(2'-methoxy-6'-methylcyclohex-2' en-4'-one) (griseofulvin)	30	19	100	37	41
Methylreserpate 3,4,5-trimethoxy-benzoic acid ester (reserpine)	10	0	96	36	75
Trifluoroperazine dihydrochloride (stelazine)	2	0	82	71	0
1 naphthyl n-methyl carbamate (carbaryl)	0.2	10	100	166	0

* Adjusted for control mortality and sterility by Abbot's formula.

Table 8. The sterilising effect of 8 juvenile hormone analogues against female _Diparopsis_ applied by injection.

Juvenile hormone analogue	Dose (ug)	% 24 h. mortality*	% 7 day mortality*	Mating as percentage of control mating	oviposition as percentage of control oviposition	% sterility*
Methyl farnesoate dihydrochloride (DMF)	1000	0	15	15	32	46
Farnesyl diethylamine	500	64	90	24	108	39
Farnesyl methyl ether	500	14	77	20	56	75
Ethyl farnesoate	100	0	48	28	96	0
10,11-epoxymethyl farnesoate	100	0	0	105	66	23
Farnesol	100	0	14	75	95	1
Farnesenic acid	100	0	50	61	143	9
Farnesal +	100	0	6	105	94	19

* Adjusted for control mortality and sterility by Abbot 's formula.

+ applied topically in acetone.

Table 9. To show whether treatment with juvenile hormone analogues affects the pattern of egg laying and fertility on successive mating days of mated female red bollworm adults.

		MATING DAYS															
Sample	Treatment (µg)	2			3			4			5			6		
		Eggs laid	Hatch	% hatch	Eggs laid	Hatch	% hatch	Eggs laid	Hatch	% hatch	Eggs laid	Hatch	% hatch	Eggs laid	Hatch	% hatch
DMF	1000	6	0	0	24	24	100	0	0	-	11	2	18	0	0	-
	500	24	0	0	133	106	80	206	251	88	253	179	71	162	114	70
	100	48	39	81	108	104	96	33	31	94	89	27	30	62	61	98
	control	85	84	94	149	144	97	165	159	96	206	186	90	210	159	76
Farnesyl diethyl-amine	500	0	-	-	78	56	72	114	69	61	114	67	59	72	18	25
	100	150	154	98	123	122	99	170	151	89	171	152	89	66	37	56
	control	188	182	97	227	221	97	346	336	97	347	336	97	289	201	70
Farnesyl methyl ether	500	0	-	-	0	-	-	16	7	44	26	4	15	15	1	7
	100	123	114	93	112	82	73	48	43	90	151	121	80	118	59	50
	control	6	0	0	51	33	65	96	25	26	102	150	82	293	203	69
10,11-epoxymethyl farnesoate	100	103	90	87	317	303	96	301	265	88	306	247	81	284	209	74
	control	419	377	82	492	473	96	401	368	92	409	291	71	406	392	97
Ethyl Farnesoate	100	162	156	96	136	135	99	31	26	84	43	39	91	39	36	92
	50	155	130	84	103	102	99	65	64	99	31	31	100	0	0	-
	20	28	23	82	90	90	100	59	46	78	65	20	36	31	10	32
	control	205	201	98	249	213	86	252	213	35	109	153	81	123	102	83

(B) Secondary evaluation.

1. Estimation of the median sterilising and median lethal doses of chemosterilants active against Diparopsis adults.

The results from the probit analysis of the dose response data shown in Fig. 8 and appendix 1 showed that in all instances, with the exception of hemel, a linear relationship existed between the concentration (log x) and the percentage sterility induced and the mortality (probit Y). The regression equations and the calculated ED_{50} are shown in Table 10. A comparison of the heterogeneity (X^2) about the regression lines fitted with and without the constraint of parallelism gave no evidence that they were not parallel. This made it possible to calculate the ratio between sterilising and toxic doses of the applied chemosterilants (Table 11). 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 sterility index value shown in Table 11 is at the ED_{50} level only.

Clearly a large sterility index indicated, a wide safety margin between sterilising and lethal doses

In males the greatest sterility index of 24.0 was shown by apholate, followed by tepa with 17.9 and metepa with 11.5.

Fig. 8(a) Probit regression lines for sterility and mortality induced

by tepa against adult Diparopsis. (A) injecting male moths,

(B) injecting female moths, (C) topical treatment of male

moths, ▌ = sterility values, 0 = mortality values.
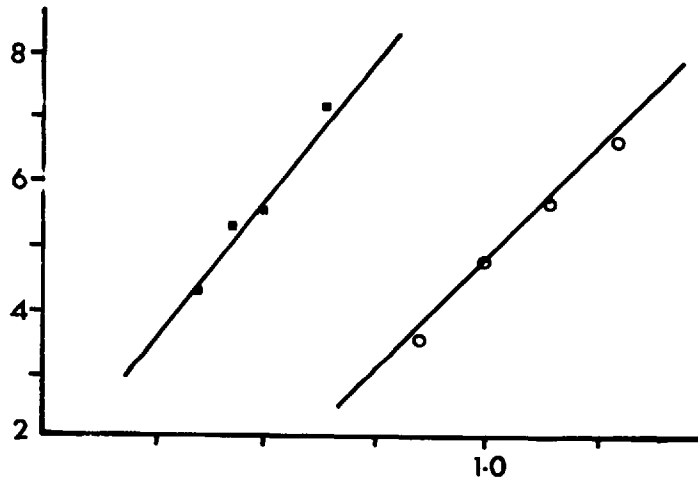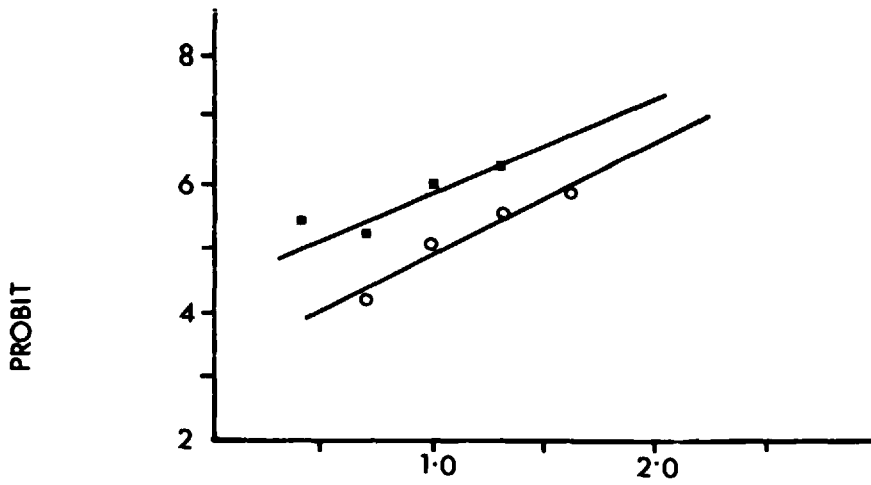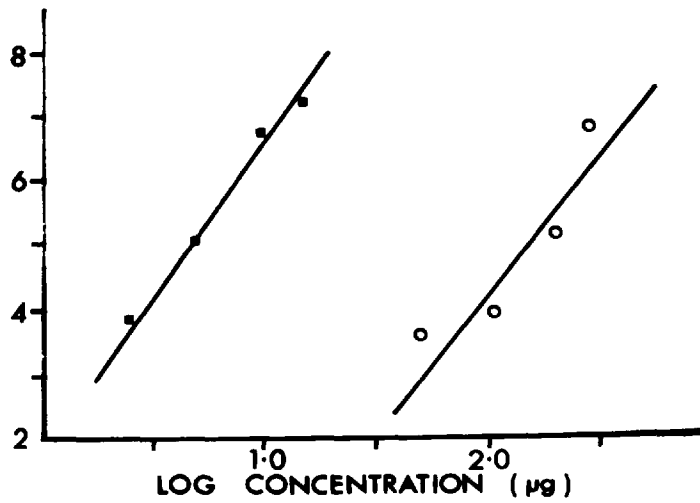
Fig. 8(b) Probit regression lines for sterility and mortality of certain

chemosterilants against adult male <u>Diparopsis</u> (A) apholate,

(B) metepa, (C) hempa and (D) triphenyl tin acetate,

█ = sterility values, 0 = mortality values.
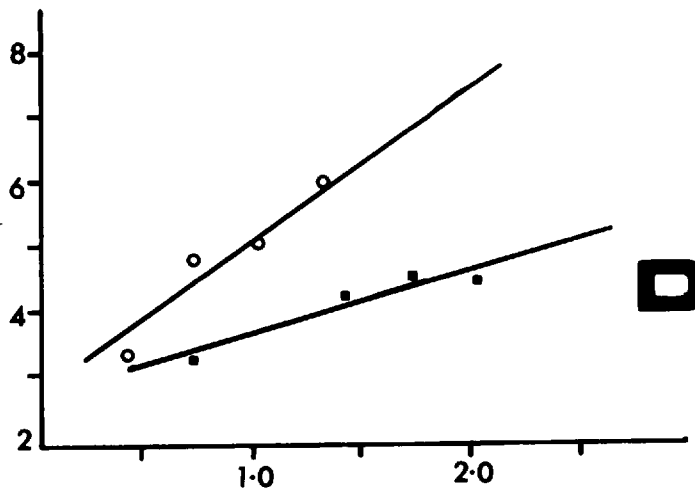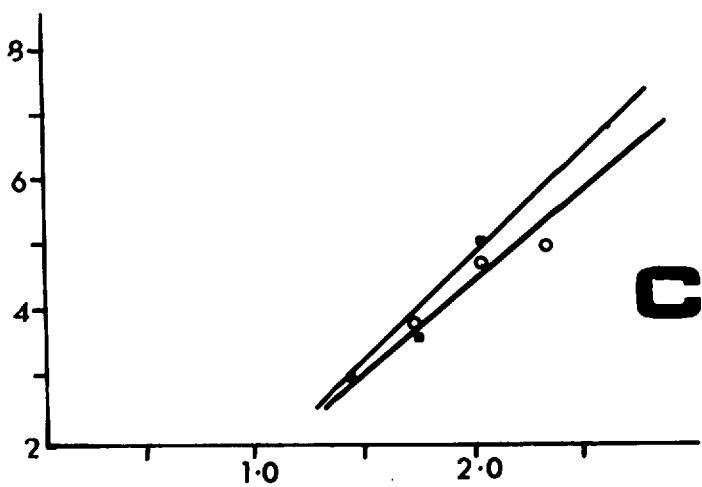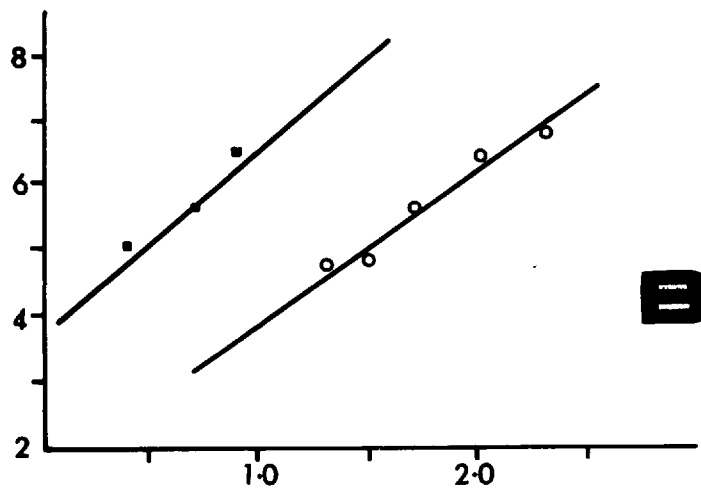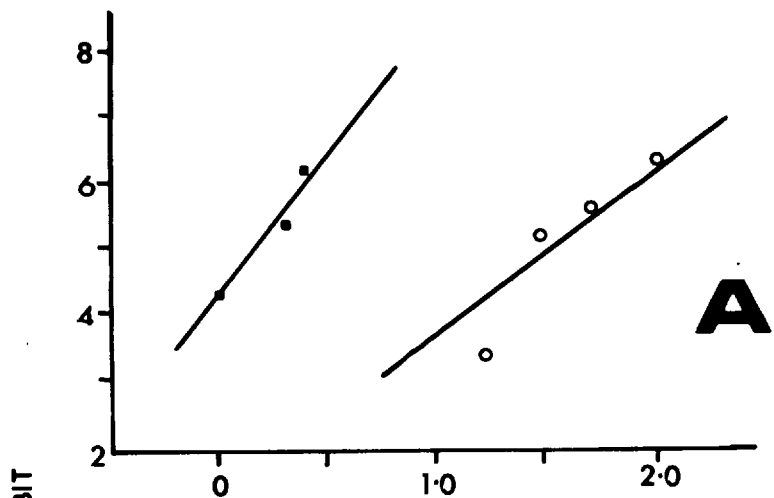
Table 10. Sterility and mortality regression equations and calculated ED_{50}; of certain chemosterilants against (A) _Diparopsis_ moths when applied by injection (B) when tepa applied topically to male moths.

Chemosterilant	Sex treated	STERILITY				MORTALITY			
		Regression equation	Log factor	ED_{50} (µg)	95% fiducial limits	Regression equation	Log factor	ED_{50} (µg)	95% fiducial limits
A. tepa	males	Y=1.17+4.47x	10	0.73	0.67-0.78	Y=1.40+3.37x	1	11.7	8.6-15.9
tepa	females	Y=4.42+1.42x	1	2.56	1.83-3.58	Y=3.26+1.63x	1	11.7	8.0-17.1
apholate	males	Y=-0.13+4.44x	10	1.5	1.44-1.66	Y=1.39+2.41x	1	31.5	20.1-49.3
metepa	males	Y=3.68+2.93x	1	2.8	2.10-3.77	Y=1.65+2.28x	1	29.5	21.3-40.8
hempa	males	Y=-1.10+3.04x	1	101.5	77.8-132.6	Y=0.76+2.67x	1	143.6	112.1-184.0
triphenyl tin acetate	males	Y=2.76+1.01x	10	16.1	4.90-53.20	Y=2.84+2.33x	1	8.5	5.9-12.2
B tepa	males	Y=1.77+4.78x	1	4.74	4.70-4.78	Y=0.47+2.23x	1	105.4	81.3-143.0

Table 11. Sterility indices (LD_{50}/SD_{50}) of (A) certain chemosterilants applied to <u>Diparopsis</u> adults by injection: (B) tepa applied topically to male moths and by injection to female moths.

	Chemosterilant	Method of treatment	sex treated	Sterility index	95% fuducial limits	X^2 value for parallelism of regressions
A.	Tepa	injection	males	17.9	10.1-30.7	1.05
	Apholate	"	"	24.0	13.1-43.7	3.75
	Metepa	"	"	11.5	6.6-19.9	1.58
	Hempa	"	"	1.4	0.1-1.9	0.22
	Triphenyl tin acetate	"	"	-7.7*		4.43
B.	Tepa	injection	females	4.2	2.8-6.4	0.24
	Tepa	topical	males	34.6	26.1-45.9	0.73

* At ED_{50} values only.

For these substances the calculated 50% sterilising doses
(SD_{50}) were 1.5, 0.73 and 2.8 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 Diparopsis.

A low sterility index of 4.2 was obtained with tepa-
treated females, which again suggests that mating would be
adversely affected at doses causing complete sterility.
This conclusion was confirmed by mating experiment (Table 12).
Treatment of females with tepa also caused a significant
reduction in the rate of oviposition (Table 13).

2. Competitive mating tests.

The results of competitive mating tests for a single
mating night following injection application of tepa at the
SD_{95} level are presented in Table 14. The results showed the
treated males to be fully competitive in that the expected
percentage of sterile matings in each instance approximated
to the theoretical value $(X^2 = 0.61)$. A single test with
apholate at the SD_{95} level of treatment (Table 14) likewise
showed the sterilised male insects to be fully competitive when
exposed to one mating night only.

Table 12. The effects of tepa injections on the mating of Diparopsis.

Dose (μg)	Mating as percentage of control mating*	
	Treated females x normal males[+]	Treated males x normal females[+]
80	0	0
40	19.5	24.9
20	75.6	37.5
10	61.0	85.0
5	48.7	60.0
2.5	-	106

* Minimal mortality at all dose levels for the 2 day mating period

+ Data based on 20 replicates at each concentration

SD_{95} for female moths 30.9 μg. SD_{95} for male moths 1.3 μg.

Table 13. Effect of tepa treatment by injection on the rate of oviposition in *Diparopsis* females mated with untreated males (4 day oviposition period).

Dose (µg)	No. of mated females	No. of eggs	Mean no. of eggs per female	U value	Significance of oviposition reduction compared to control p
40	2	7	3.5	2	0.025
20	14	369	26.6	43	0.01
10	21	486	26.4	142	.006
5	29	1109	38.2	239	0.42
2.5	3	122	40.6	23	
C	16	659	41.2		

Table 14. Competitiveness of male <u>Diparopsis</u> treated by injection with tepa and apholate at the SD$_{95}$ level.

Chemosterilant	Mating ratio T♂ U♂ U♀	No. of moths released	Age of males (days)	Age of females (days)	% mating of recovered females	Total matings	No. fertile	No. sterile	% sterile mating* Expected	% sterile mating* Actual
Tepa	1: 1: 1	42:42:42	4	1	73.6	28	14	14	50	50
	1: 3: 1	24:72:24	5	3	91.5	21	16	5	25	23.8
	1: 5: 1	10:50:10	1	1	100	8	6	2	16.7	25
	9: 1: 1	90:10:10	2	1	100	10	2	8	90	80
Apholate	1: 1: 1	42:42:42	4	2	51.5	18	8	10	50	55.6

* Frequency of sterile matings compared with fertile matings using tepa;

X^2=0.61 indicating no significant heterogeneity.

3. Repetitive mating tests.

The two most effective chemosterilants for male moths were further evaluated by repetitive mating tests; the results are shown in Table 15 and clearly indicated tepa to be the most effective chemosterilant. The mating frequency approximated closely to the control mating frequency ($X^2 = 0.5$), whereas the mating frequency after apholate treatment was reduced ($X^2 = 19$). The results also show 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 at the SD_{95} level was permanent (Table 16).

4. Tepa treatment of Diparopsis moths by topical application.

The regression lines following topical application of graded concentrations of tepa again showed that a linear relationship existed between doses of chemosterilant and percentage mortality and sterility (Table 12 Fig. 8). The regression lines were parallel ($X^2 = 0.73$) and therefore a sterility index of 34.6 was calculated with an SD_{50} value of 4.74 µg (Table 11).

The results of competitive mating tests are shown in Table 17. The frequency of sterile mating compared with fertile mating ($X^2 = 3.65$) gave no evidence for significant heterogeneity and it was concluded that mating was not adversely affected as

Table 15. Mating frequency and mean mortality of male _Diparopsis_ in repetitive mating tests after treatment by injection with estimated ED_{95} sterilising doses of tepa and apholate.

Treatment	No. of males treated	Mean mortality (days)	Mean no. matings per male	Mating frequencies				X^2 values for heterogeneity of mating frequency	P
				X0	X1	X2	X3		
Control	37	4.6	1.2	9	15	11	2	0.5	>.05
Tepa	33	4.0	1.0	9	15	8	1	19.0	<.001
Apholate	39	3.1	0.5	21	17	1	0		

Table 16. Permanence of sterility in male _Diparopsis_ injected with tepa at ED_{95} sterility level (25 males per treatment).

Time of mating in days post-treatment	No. mating with 1 day old females	Total eggs laid	No. hatching	% hatch
1	13	1401	3	0.2
2	14	1542	0	0
3	9	695	0	0
4	4	352	24	7
5	6	346	12	3.5
6	4	330	0	0
Total		4666	39	0.84

Table 17. Competitiveness* of male _Diparopsis_ treated topically with 15 µg of tepa.

No. of replicates	Total eggs from fertile matings	Total eggs from sterile matings	Mean eggs per fertile mating +	Mean eggs per sterile mating +	Total matings	No. fertile	No. sterile	% sterile mating Expected**	Actual
99	2690	1110	104	62	44	26	18	50	40.9
97	1993	559	71	40	42	28	14	50	33.3
91	2067	850	115	57	34	18	15	50	45.5
73	2699	686	117	98	31	23	7	50	23.3
39	1223	758	153	126	14	8	6	50	42.8

* In all replicates one treated male, one normal male and one normal female were enclosed together. Matings were confirmed by dissection of females for spermatophores at the end of the test period.

** Frequency of sterile matings compared with fertile mating $X^2=3.65$, indicating no evidence for significant heterogeneity.

+ Comparison of mean fecundity of females mated with sterile or fertile females $X^2=10.95$ indicating significant heterogeneity at 1% level.

-75-

a result of treatment. A comparison of the mean fecundity
of females mated with sterile males compared with those mated
to fertile males (X^2 = 10.95) 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 sterile and fertile males when mated
on successive days post-treatment is shown in Table 18.
The results suggested that shortly after treatment some
incapacitation of sterilised males occurred, although the
insects soon completely recovered.

5. Sterilising effect of the chemosterilant
   tepa against male Autographa.

During the course of many mating experiments it was noted
that female Diparopsis generally mated only once. The
sterilising effects of injected doses of tepa against a
multiple mating moth Autographa gamma L. were also briefly
examined and the results shown in Table 19.
A sterility level of 99% was achieved in male moths at an
injected dose of 10 μg. Subsequent examination of the female
moths for spermatophores indicated that in some instances
several matings has occurred.

Table 18. Competitiveness of male _Diparopsis_ of successive days following topical treatment with 15 µg of tepa.

Days post-treatment when mated	Total matings	Fertile matings	Sterile matings	% sterile matings	
				Expected *	Actual
1	38	31	7	50	18.5
2	41	27	14	50	34.1
3	31	16	15	50	48.4
4	26	15	11	50	42.4

* Frequency of sterile mating compared with fertile mating $x^2=7.81$, which indicates heterogeneity at 5% level.

Table 19. The sterilising effect of injected doses of tepa on male _Autographa_ moths relative to mating performance and mortality.

Treatment (µg)	No. treated	Mean male mortality (days)	% mating	Mean no. of spermatophores per mated female	Eggs laid mated females	Mean no. eggs laid per mated female	% egg hatch
Control	10	3.4	50	0.8	1654	330.8	60.0
5	10	8.7	80	2.1	3101	387.6	13.8
10	10	4.1	100	1.0	3237	323.7	0.98

DISCUSSION

Primary evaluation of the various classes of chemosterilants showed the aziridine alkylating agents to be the most effective sterilising agents particularly against male moths. The sulphonic acid alkylating agent busulphan was ineffective. Members of this group were also inactive when tested against another Lepidopteron Heliothis virescens when applied either topically or orally (Flint et al 1968a, 1968b) and also against the parasitic wasp Bracon hebetor (LaChance & Leverich, 1969).

The chemosterilant activities of selected phosphoramides and s-triazines against other insect species are summarised in Tables 20 and 21. The phosphoramides listed were active against several insect species but usually at high doses. The boll-weevil Anthonomis grandis and the tobacco budworm Heliothis virescens were however completely unaffected. Two of the phosphoramides listed, bis (dimethylamino)piperodinophosphine oxide and bis(dimethylamino) morpholino phosphine oxide (BDMPO) were reported to be active against the boll-weevil and inactive against the housefly and the red bollworm; while hemel, the most active of the s-triazines against the red bollworm showed limited sterilising action against codling moth, housefly and pea aphid and was inactive against the boll-weevil.

Table 20. A survey of the chemosterilant activity of phosphoramides when applied to adult insects.   (a) Sterilising activity: High (++), low (+), nil (-).

Insect order	Species name	Common name	Phosphoramide chemosterilant	Sex treated	Method of application	Activity[a]	Reference
Diptera	Musca domestica	housefly	hempa	male	injection	++	Torry & Borkovec (1965)
				male	topical	+	"
				both sexes	oral	++	"
			bis(dimethylamino) piperidine oxide	both sexes	oral		Borkovec (priv. comm.)
			bis(dimethylamino) morpholine phosphine oxide	both sexes	oral	-	"
			hydroxyhempa	both sexes	oral	++	Borkovec (priv. comm.)
	Musca domestica vicina	oriental housefly	hempa	male	oral	++	Hafez & Osman (1969)
	Cochliomyia hominivorax	screw-worm	hempa	both sexes	oral	+	Terry & Borkovec (1965)
	Anastrepha ludens	Mexican fruit fly	hempa	male	oral	+	Terry & Borkovec (1965)
	Anstrephus cucumis	cucumber fly	hempa	both sexes	oral	+	Hooper (1969)
	Ceratitis capitata	medfly	hempa	both sexes	oral	-	Orphanidis & Iatsakos (1969)
	Dacus oleae	olive fly	hempa	both sexes	oral	+	Orphandis & Iatsakos (1969)
	Hippelates collusor	eye-gnat	hempa	both sexes	oral	++	Hulla (1968)
Coleoptera	Anthonomus grandis	boll weevil	hempa	male	oral	-	Klassen et al (1968)
			bis(dimethylamino) piperidine oxide	male	oral	++	Klassen et al (1968)
			bis(dimethylamino) morpholino phosphine oxide	male	oral	+	Klassen et al (1968)
	Popillia japonica	Japanese beetle	hempa	both sexes	topical	+	Ladd (1970)
			thiohempa	both sexes	topical	++	Ladd (1970)
	Callosobruchus chinensis	Azuki bean weevil	hempa	both sexes	topical	++	Nagasawe, Shinohara & Shiba (1966)
			thiohempa	both sexes	topical, dipping and contact	++	Borkovec et al (1968)
Hymenoptera	Bracon hebetor	parasitic wasp	bis(dimethylamino) piperidine oxide	male	contact	-	LaChance & Leverich (1969)
Lepidoptera	Carpocapsa pomonella	codling moth	hempa	both sexes	topical	+	Terry & Borkovec (1965)
	Heliothis virescens	tobacco budworm	hempa	both sexes	topical & oral	-	Flint et al (1968a & 1968b)
			bis(dimethylamino) morpholino phosphine oxide	both sexes	topical & oral	-	"
			bis (dimethylamino) morpholino phosphine oxide	both sexes	topical & oral	-	"
Hemiptera	Oncopeltus fasciatus	weed bug	hempa	males	oral	+	Abbot (1966)
				males	topical	-	Economopoulos & Gordon (1969)
	Acyrthosiphum pisum	pea aphid	hempa	both sexes	oral	-	Bhalla & Robinson (1968)
Orthoptera	Blatella germinica	German cockroach	hempa	both sexes	oral	+	Hopper (1969)

Table 21. A survey of the chemosterilant activity of s-triazines applied to adult insects

Insect order	Species name	Common name	s-triazine chemosterilant	Sex treated	Method of application	Activity[a]	Reference
Diptera	Musca domestica	housefly	hemel	both sexes	topical & oral	++	Borkovec & Terry (1965)
			hydroxyhemal	both sexes	oral	++	Borkovec (priv. comm.)
			trimethylol melamine	both sexes	oral	+	Borkovec & Terry (1965)
			2,4-diamino-6-(2-furyl) s-triazine	both sexes	oral	++	Borkovec (1966)
			2,4-diamino-6- morpholino -s-triazine	both sexes	oral	++	Borkovec & Terry (1965)
	Anastrepha ludens	Mexican fruit fly	2,4-diamino-6-morpholino -s-triazine	both sexes	oral	–	Borkovec (1966)
	Ceratitis capitata	medfly	hemel	males & females	oral	+	Orphanidis & Patsakos (1969)
	Dacus oleae	olive fly	hemel	males & females	oral	+	Orphanidis & Patsakos (1969)
	Cochliomyia hominivorax	screw worm	2,4-diamino-6-(2furyl) s-triazine	both sexes	oral	–	Borkovec (1966)
	Drosophila melanogaster	fruit fly	trimethylol melamine	both sexes	oral	+	Röhrborn (1962)
Coleoptera	Anthonomus grandis	boll weevil	hemel	male	oral	–	Klassen et al (1968)
			2,4-diamino-6-morpholino -s-triazine	male	oral	–	Klassen et al (1968)
	Popillia japonica	Japanese beetle	hemel	both sexes	topical	–	Ladd (1970)
			2,4-diamino-6-(2-furyl) s-triazine	both sexes	oral	+	"
			N^2,N^2-diethyl melamine HCl	both sexes	dipping	++	"
Lepidoptera	Carpocapsa pomonella	codling moth	hemel	both sexes	topical	++	Borkovec & Terry (1965)
Hemiptera	Acyrthosiphum pisum	pea aphid	hemel	both sexes	oral	–	Bhalla & Robinson (1968)
			2,4-diamino-6-morpholino -s-triazine	both sexes	oral	–	Bhalla & Robinson (1968)

(a) Sterilising activity: High (++), low (+), nil (–)

No satisfactory explanation is available to account for the apparent specificity of these chemosterilants. This problem will be discussed at greater length in Part V.

The candidate chemicals tested against Diparopsis were applied in a single injected or topical dose and higher activity may have been shown if alternative methods of application had been selected. Chamberlain (1962) and Gouk et al (1963b) showed that the method of application at least affected the induction of side-effects. Crystal (1964b) concluded that for aziridines the multiple oral method was far superior for monofunctional and bifunctional compounds, while topical application was better than feeding for poly-functional groups. Similarly a single feeding of apholate failed to sterilise adult male fall army worms Spodoptera frugiperda (J.E. Smith), even though the amount consumed exceeded that required by continuous feeding (Young & Cox, 1965).

In the present study egg-hatch was taken as the criterion as to whether sterility had been induced. It is noted however, that Borkovec (1966) and LaBrecque et al (1968) showed that some of the s-triazine chemosterilants exerted their maximum effect against houseflies by suppressing imaginal development at the pupal stage. A similar effect was noted by Downey (1968) when larvae of gypsy moth were treated with hempa; such delayed activity would therefore not have been detected. Given these assay limitations it is evident that only the aziridines

tepa, apholate and metepa were sufficiently active to warrant more detailed examination. When efficacy was expressed as the ratio between sterilising and lethal doses it was shown that apholate and tepa were significantly better candidates than metepa. The repetitive mating tests, however, clearly indicated tepa to be the most 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 suggested that the release of mass-reared or mass-collected moths would be feasible for a sterile-male release programme.

The possibilities for practical control measures involving the use of chemosterilants are considered in Part II.

PART II.

Possible practical control procedures involving the use

of chemosterilants.

INTRODUCTION AND REVIEW OF LITERATURE

In part I the chemosterilant tepa was shown to be highly effective in sterilising _Diparopsis_ male moths. Unfortunately tepa, together with related compounds is not only toxic but can cause sterility and zygote abnormalities in mammals (Barnes, 1964; Gaines & Kimborough, 1964; Hayes, 1964; Epstein _et al_, 1970), while chemicals of this class can efficiently break human chromosomes (Hampel & Gerhartz, 1965).

Holmson & Leasure (1966) found that tepa inhibited the growth of grasses, while Jalil & Morrison (1969) showed it to be phytotoxic when sprayed onto leaves of scarlet runner _Phaseolus coccineus_ at a concentration of 0.05%. McGovern _et al_ (1969) similarly found apholate to be phytotoxic when sprayed onto cotton plants. It is therefore evident that the widespread contamination of the environment by such compounds would be highly undesirable.

Chemosterilants such as tepa might be used with comparative safety as an alternative to radiation for sterilising mass-reared insects. This procedure was adopted with the Mexican fruit fly _Anastrepha ludens_. Tepa-sterilised flies were used to provide an efficient barrier to female fruit flies invading Southern California from Mexico (Steiner, 1965). Tepa-sterilised mass-reared houseflies were released in the island of Volano located off the coast of Italy in order to eradicate the insect

after first reducing the wild fly population with Vapona (DDVP) strips (Saccà et al, 1967). A rather bizarre approach, suggested by Morgan (1967), was the release of 'booby trapped' females. Small chamois pads treated with metepa were attached to the abdomens of female houseflies, in order that males attempting copulation would sterilise themselves.

An even more ingenious and certainly more practical suggestion was made by Whitten & Norris (1967). They proposed that resistant strains of insects to a particular insecticide should be sterilised, loaded up with that particular insecticide and released. In the laboratory they showed that individual female Lucilia cuprina surviving 0.5 µl of 2% dieldrin exposed to sensitive males killed up to 100 through contact during attempted mating. However where attempts have been made to directly sterilise the natural population with chemosterilants, the use of various baits has been adopted, in order to minimise hazards, although even the advisability of this approach has been questioned by Ascher (1967).

The effectiveness of direct spray application of apholate for the control of boll-weevil on cotton plants under strictly controlled and isolated conditions was shown by McGovern et al (1969). This demonstrated the potential of the method should safer chemicals be eventually discovered.

Sugar baits containing tepa, metepa and apholate were used against relatively isolated populations of houseflies

on rubbish dumps or poultry farms. In general considerable
reductions in insect numbers occurred (LaBrecque et al, 1962b ,1963,
Gouk et al 1963; Mathis & Schoof, 1965; Sacca & Stella, 1964).
Experiments on a larger scale were carried out on certain
West Indian Islands (Mcifert et al, 1967). On one island, 1%
metepa liquid bait applied over an 18 month period induced
more than 80% sterility in the housefly population, although
eradication was not achieved. At the other end of the scale
Hansens (1965) considered the effect of a chemosterilant on
large-cage populations of houseflies. He found that a sugar bait
containing 2.0% of apholate reduced the population by only
57% in the 12 week test period, while an insecticide bait of
trichlorphon eliminated almost the entire population in one
week. Other large cage experiments with houseflies were carried
out by Ratcliffe & Ristich (1965). They evaluated various
formulations of apholate and related chemosterilants and found
that effectiveness was associated with good attractancy and
ready availability of the sterilant; granular sugar baits
being particularly effective. This work emphasises the importance
of preliminary cage experiments before field studies are
undertaken. Indeed, Sacca, Magrone & Scirocchi (1965) showed
that metepa had repellant properties for houseflies. Luckmann
et al (1967) used apholate bait stations for large cage tests
for control of Onion maggot Hylema antigua (Meigen) when more
than 89% sterility and insect control was obtained. Mason & Smith
(1968) used fermenting baits in glass jars coated with apholate

for the possible control of _Drosophila melanogaster,_ on small tomato plots, but without success. Sanchez-Rivello & Shaw (1966) used aqueous tepa solutions in conjunction with protein hydrolysate lure contained in plastic bait stations for the control of Mexican fruit fly _Anastrepha ludens,_ in an isolated mango grove. The initial results were promising, although further work has been discontinued. Orphanidis _et al_ (1966) reported the use of bait stations containing 0.4% of apholate in an isolated olive grove for the control of olive fly _Dacus oleae._ The egg hatch of captured females in the treated grove was halved compared with those from a control grove. Novák, Landa & Řezábová (1968) considered the possibility of controlling the forest pest _Hylobius abietus_ by means of baited plastic traps, using apholate at a concentration of 2.0%; but this was ineffective in reducing the local population. Howland, Vail & Henneberry (1966) experimented with the self-sterilising technique using the cabbage looper moth _Trichoplusia ni._ The bait consisted of a 15 watt black-light enclosed in a tepa coated cellulose nitrate cylinder. These units installed in cages with 30-60 pairs of untreated moths, reduced subsequent larval populations by an average of 80%. Grant _et al._ (1970) reported the development of traps to which male mosquitoes _Culex fatigans,_ could be attracted either by light or specific baits. Using a battery operated trap fitted with U.V. light, adults were drawn into a chamber treated with tepa. The escaping insects showed

87-95% sterility.

Adult _Diparopsis_ are also attracted to light and much early work was devoted to attempts to control the insect by light-traps, although none of these were successful (see review by Pearson, 1958). Since this work was reported much more efficient light traps have been developed as potential insect control or scouting agents and considerable effort made in both the design and output of such light traps (Hollingsworth _et al_, 1968; Sparks _et al_, 1967; Stewart _et al_, 1969).

A sex attractant or even the caged virgin female either alone or in combination with a light source was an effective lure for the tobacco-bud worm _Heliothis virescens_ (F.) (Hendricks, 1968) and cabbage looper _Trichoplusia ni_ (Debolt, 1970).

Tunstall (1965) has shown that _Diparopsis_ has a sex attractant while the isolation, identification and eventual synthesis of the pheromone is being investigated (Moorhouse _et al_, 1969). Preliminary observations made by Campion (1967a) suggested that male _Diparopsis_ probing on aqueous solutions of tepa would be sterilised. The main aim of this series of experiments was to confirm these observations and to examine the possibilities of autosterilisation by means of a bait station using virgin females as the lure and tepa as the chemosterilant. By recourse to simple population models the feasibility of such an approach in the field was determined. The practicability of a conventional sterile release method was also considered.

MATERIALS AND METHODS

1. Contact sterility methods.

One day old male moths were exposed to filter paper
saturated with graded concentrations of aqueous tepa, contained in
plastic sandwich boxes (11 x 6 x $3\frac{1}{2}$ in) for varying lengths of
time. The insects were then mated to virgin females in the
manner described in Part I.

Vegetable oils were applied in 1 µl amounts to the ventral
abdominal surface of male moths, following the procedure
described in   Part I and then mated as before in the standard
manner.

2. Probing experiments.

The moths were placed in contact with filter paper
saturated with aqueous solutions of tepa or sugar and closely
observed. If no probing occurred within 1 minute the insect
was removed. If feeding commenced then in some instances the
total probing time was noted, or on other occassions probing
was limited to 5 minutes. The amount of liquid imbibed was
determined by weighing the insect before and after probing..

The sterilising effect of tepa following probing of the
male moths was determined in the standard manner, described in
Part I.

3. <u>Repetitive and competitive mating tests.</u>

After exposure to tepa treated surfaces the sexual
vigour of the sterilised insects was determined by the
repetitive or competitive mating tests described in Part I.

4. <u>Probing and fat body depletion.</u>

Groups of 40 male moths of known age were confined in
plastic sandwich boxes (11 x 6 x $3\frac{1}{2}$ in). They were held either
under constant light or a 12 hour photoperiod at 25-27°C.
20 moths from each group were removed each day and given the
opportunity to probe on wet filter paper for 1 minute. The moths
were then dissected and the state of fat body recorded as one
of four categories (++++ = considerable fat body to + = virtually
no fat body).

5. <u>Effects of drinking water, cane sugar and honey
water on the longevity of male moths.</u>

Groups of 25-40 one day old male moths were kept in
plastic sandwich boxes at 27°C and 70% R.H. and a 12 hour
photoperiod. Four small cotton wool pads contained in
suitable plastic holders were saturated with either tap-water,
10% cane sugar or 1% honey, were placed in each box and
replenished each day. Control moths were without either water
or sugar water. Daily mortality counts were made and approximate

ED_{50} and ED_{90} values were calculated by constructing eye-fitted probit lines.

6. Selection of a marker dye.

To determine the rate of visitation of male moths to a bait station, a marker dye was needed which would not only mark externally, but also stain the gut or other internal organs and be retained for several days following probing. The use of various dyes for marking insects' was reviewed by Southwood (1966). Subsequently Heron (1968) used vital dyes for behavioural studies of the larch sawfly while Showers et al (1968) used Calco oil red to stain the European corn-borer Ostrinia nubilalis. However, these workers incorporated the dye into the larval diet. Little information exists for marking insects by the direct uptake of dye in the adult stage.

Aqueous solutions of the dyes listed in Table 32 at concentrations of 0.1% and 0.5% were prepared. Suitably thirsted male moths were then placed in contact with filter paper saturated with dye solution and allowed to probe in the manner previously described. Groups of five moths were dissected under the binocular microscope after intervals of 1, 3 and 5 days and the distribution and persistence of the dye noted.

7. Bait station experiments.

For the bait station experiments, a cage was constructed of tygan mesh, mounted on a dexion frame measuring 36 x 36 x 40 in,

housed in a constant temperature room at 25°C and uncontrolled humidity. The humidity was however noted at the beginning and end of each experiment by means of a paper hygrometer. The bait station was constructed of galvanised metal (Fig. 11) and placed in the centre of the cage floor. It was baited with varying numbers of virgin females. The funnel of the bait station was lined with blotting paper saturated with an aqueous solution of the marker dye and kept moist by a cotton wool wick connected to a reservoir of dye solution. A known number of males contained in an 11 x 6 x $3\frac{1}{2}$ in plastic box were released into the cage at 5-5.30 pm. They were maintained in complete darkness until 9-930am. the following morning by which time the moths were generally well dispersed throughout the cage. The males were then collected individually in glass specimen tubes and examined under the binocular microscope externally for traces of dye indicating whether they had visited the station and internally to see whether active probing had occurred.

In assessing the relative attractiveness of increasing numbers of virgin females in the lure holder of the bait station, the statistical significance of the results was determined by calculation of the standard error of attraction.

8. The response of tepa-treated males to virgin females in a bait station.

Male moths of known age were sterilised by overnight contact

with filter paper saturated with 0.2% aqueous tepa, while an
equal number of untreated males of similar age were left in
contact with filter paper saturated with water. Both sets of
males were then transferred to dry plastic boxes (11 x 6 x $3\frac{1}{2}$ in)
and the control males marked. On successive nights following
treatment an approximately equal number of treated and untreated
males were released into the bait station cage, previously
described and the lure holder baited with three virgin females.
The males were collected the following morning and examined
in the standard manner for traces of dye as evidence that a
visit to the bait station had occurred. Mortalities following
the overnight release were also recorded.

9. Population models.

In an attempt to approximately predict the efficiency of
caged virgin females in combination with either an insecticide
or a chemosterilant as control agents, a series of simple
population models were set up. Given a situation briefly as
follows.

There is one acre of completely isolated land containing
an overwintering population of 3,600 diapause Diparopsis pupae,
which is a conservative estimate in cotton growing areas of
Central Africa. All emerge as adults and for convenience of
calculation at a constant rate of 15 males and 15 females
each day over a period of 120 days, which is approximately
the duration of the Malawi cotton growing season. Also for the

sake of simplicity, any subsequent additions to the adult
population emerging from eggs laid as the result of fertile
matings are not considered, although the egg to adult period
is approximately 50 days. Females are considered monogamous
which is virtually correct in the case of _Diparopsis_, and mate
on the day of emergence which they can certainly do (Campion
& Outram, 1967). Except on the day of emergence 25% of the
population will die each day, as will the virgin females to be
used as attractant bait. The methods for calculating the models
were based on those described by Knipling & McGuire (1966) and
using a desk calculating machine (see Appendix 2). Other models
have been elaborated by Ailam & Galun (1967),Berryman (1967),
Lawson (1967), and Cuellar (1969).

RESULTS

1. Control and probing sterility studies.

When male moths probed on filter paper saturated with 0.1% aqueous tepa they were consistently sterilised, while similar contact periods without probing failed to cause appreciable sterility (Table 22).

Table 22. To show the importance of probing for the induction of sterility in male Diparopsis when exposed to contact with filter paper saturated with an aqueous solution of 0.1% tepa for 2-5 minutes.

Action of moth	Number of mating males	Total eggs laid	Eggs hatching	% sterility
probing	24	1017	0	100
non-probing	41	1119	855	23.6

The competitiveness of male moths sterilised by probing on tepa treated surfaces is shown in Table 23. The results indicated that the moths were fully competitive while the treatment did not reduce longevity (Table 24). Contact periods of up to 2 hours on filter paper saturated with tepa at aqueous concentrations of between 0.1 to 1.0% failed to consistently sterilise the moths (Table 25), which was presumably related to the varied probing response of the insects under treatment.

Table 23. Competitiveness of male _Diparopsis_ sterilised by probing on filter paper saturated with 0.1% aqueous tepa (after Campion & Outram, 1967).

Mating ratio T♂:U♂:U♀	No.of reps.	No. mating	% mating	Tot.eggs from fertile matings	Tot.eggs from sterile matings	%hatch from fertile matings	%hatch from sterile matings	Mean eggs per fertile mating	Mean eggs per sterile mating	No. of egg batches	No. fertile	No. sterile	%sterile** mating Expected	%sterile** mating Actual*
1:1:1	145	46	32	796	682	85	0	44.3	31.4	41	18	23	63	56
1:1:1	60	20	33.3	339	167	85	0	33.9	16.7	18	8	10	63	55.5
1:1:1	57	8	14	305	113	70	0	76.2	28.2	7	4	3	63	42.8
1:1:1	67	10	14.9	538	230	49.5	0	134.5	38.3	10	4	6	63	60.0
1:2:1	51	12	23.5	155	131	81.4	0	62.5	13.1	12	12	10	38	83.3
1:2:1	67	25	37	627	139	73.5	0	52.2	13.9	22	12	10	38	45.4
1:2:1	38	9	23.6	41	102	66.2	0	20.5	20.4	7	2	5	38	71.4
1:1:1+	38	13	34.2	295	252	30.0	0	49.2	36.0	13	6	7	63	53.8

* Adjusted for natural sterility occurring during test period.

+ Males treated by overnight contact on 0.1% tepa-water instead of usual 5 minutes probing.

** Adjusted for control sterility.

Table 24. Comparative mortalities of untreated males with males

sterilised by probing on 0.1% tepa water*.
(After Campion & Outram, 1967)

Days post-treatment	% mortality of untreated males	% mortality of treated males
3	17.2	19.3
4	40.3	36.8
7	91.5	79.0

* 57 males in each category.

Table 25. The sterilising effect on male Diparopsis when

exposed to contact with filter paper saturated with

aqueous solutions of tepa for 2 hours.

% conc. of tepa soln.	Number of males treated	Mating as percentage of control mating	Eggs laid	% sterility *
0.1	40	107	503	3.0
0.5	18	150	330	78.0
1.0	18	111	102	59.7

* Corrected for control sterility by Abbott's formula.

The probing response was shown to be related to the relative humidity to which the moths were exposed (Table 26).

The relationship between age, fat-body depletion and rate of probing is shown in Table 27. Under a 12 hour photoperiod where flight activity is stimulated, a more rapid utilization of fat body occurred, compared with quiescent moths maintained under constant illumination. On the other hand the increase in probing rate with time was virtually identical in both instances (Fig. 9).
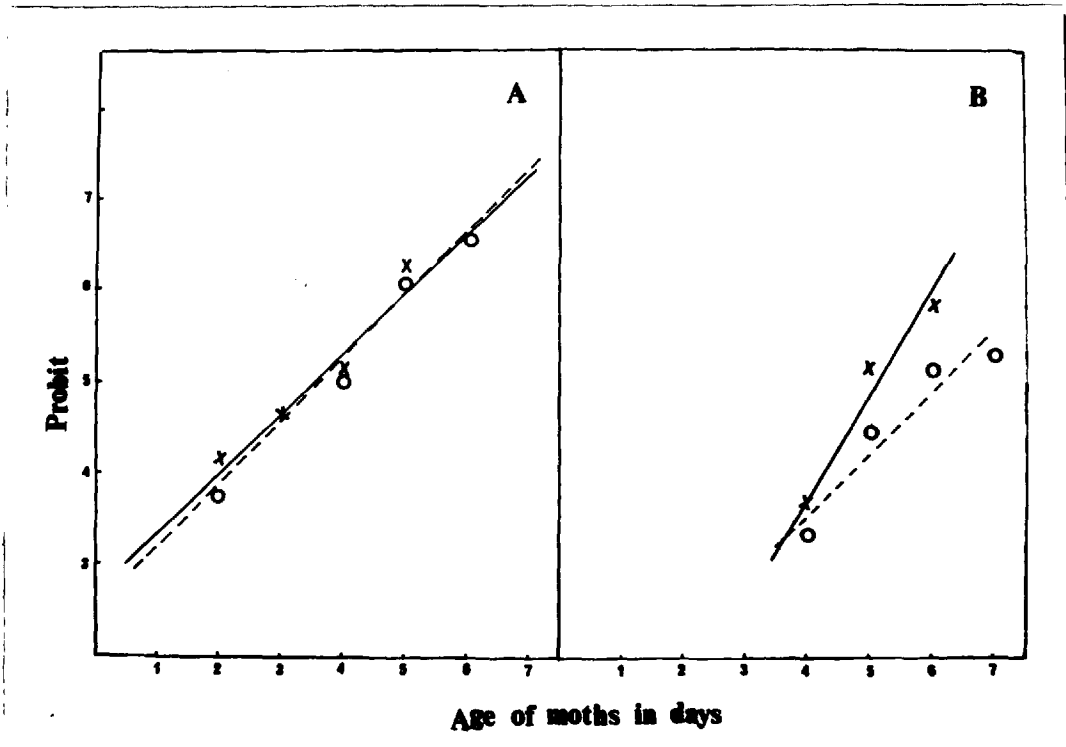
Fig. 9. Relationship between age of male Diparopsis,(A) rate of probing (B) depletion of fat body when maintained either in continuous light (inactivated) or under a 12 hour photoperiod (activated). x-x = activated moths
o-o = inactivated moths

Table 26. The relationship between dessication, probing response and amount of water imbibed by probing male _Diparopsis_. (After Campion & Outram, 1967.)

Treatment*	Whether maintained in light or darkness	No. tested	No. probed	% probed	Mean time of probing moths (min)	Mean uptake of probing moths (µl)
0% R.H.	light	32	23	72	57.5	15.5
10% R.H.	light	45	34	75.5	34.6	10.6
20% R.H.	light	48	26	54	41.0	6.5
40% R.H.	light	45	28	62	14.8	7.2
Laboratory humidity	darkness	62	25	40	20.0	5.7

* The moths were maintained at the stated humidities for 24 hours before probing assessment.

Table 27. Relationship between age of male _Diparopsis_, depletion of fat body and rate of probing. (2 x 10 moths per treatment.)

Age of moth (days)	Activated moths (12 h photoperiod)				Inactivated moths (constant illumination)			
	No. probing	% probing	No. with exhausted fat body	% with exhausted fat body	No. probing	% probing	No. with exhausted fat body	% with exhausted fat body
2	4	20	0	0	2	10	0	0
3	7	35	0	0	7	35	0	0
4	11	55	2	10	10	50	1	5
5	18	90	11	55	17	85	6	30
6	20	100	16	80	19	94	11	55
7	20	100	20	100	20	100	13	65

The effect on the mortality of male Diparopsis when provided with drinking water, 10% cane sugar, and 1% honey, compared with moths without water when maintained at 27°C, 70% R.H. and a 12 hour photoperiod is shown in Table 28.

Table 28. The effect on mortality of male Diparopsis when provided with drinking water, 10% cane sugar and 1% honey, compared with moths without water when maintained at 27°C, 70% R.H. and 12 hour photoperiod.

Treatment	No. of moths	Total mortality in days							
		1	2	3	4	5	6	7	8
No water	36	0	0	2	7	17	32	34	36
Water	28	0	0	2	3	8	12	17	22
10% cane sugar	30	0	0	2	5	6	12	22	25
1% honey	38	0	0	0	3	7	19	24	33

From eye-fitted regression lines the mortality (LD_{50} & LD_{90}) of male Diparopsis maintained at 27°C and 70% R.H. and a 12 hour photoperiod was 4.5 and 7 days respectively. When drinking water was provided the LD_{50} and LD_{90} values were increased to 6.3 and 9 days respectively, which showed some dependance on water for survival. The addition of 10% cane sugar or 1% honey to the water however made no essential difference to the longevity of the insect (Fig. 10).
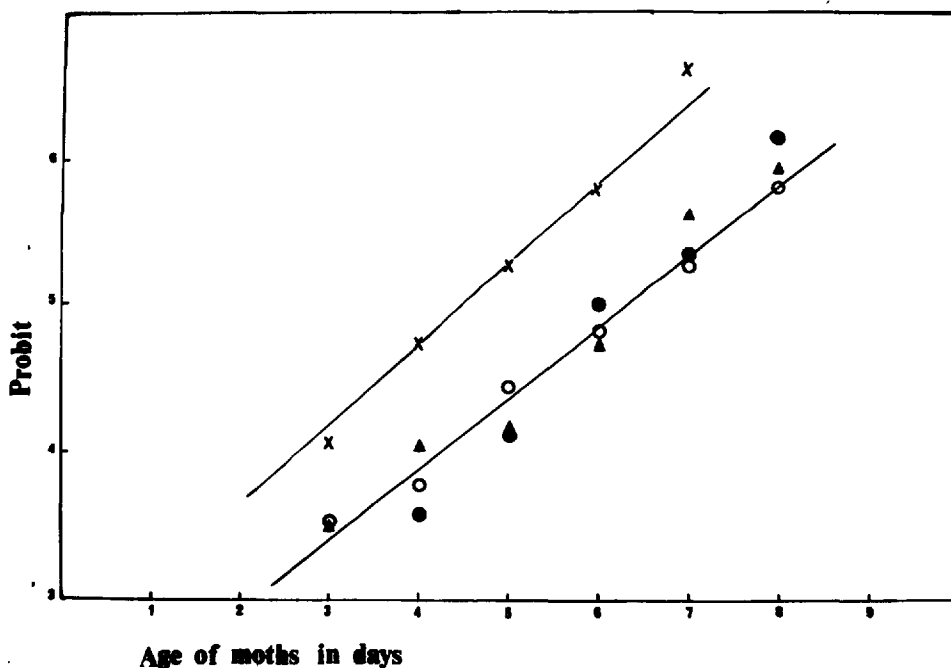
Fig. 10   Mortality of male Diparopsis, when provided with

drinking water, 10% cane sugar and 1% honey compared

with moths without water at 27°C, 70% R.H. and 12 hour

photoperiod. x-x = no water, o-o = water only,
▲-▲ = 1% cane sugar, ●-● = honey-water.

Application of a series of vegetable oils to male moths

as alternatives to water as a diluent for tepa were in general

only slightly toxic, but all reduced mating effectiveness

(Table 29).

Overnight exposure of male moths to filter paper saturated

with graduated concentrations of aqueous tepa induced a consistent

increase in the level of sterility (Table 30); from which it

was concluded that minimal probing must have occurred during

the time of exposure.

Table 29. Effect of topically applied 1 µl amounts of certain natural vegetable oils on mortality and mating performance of male Diparopsis.

Oil	No. treated	% mortality[+] 5 days post treatment	% sterile[+] eggs	% mating	Significance of mating reduction * x^2	p
tung oil (refined)	40	9.6	–	0		
olive oil (B.P.)	35	0	–	0		
ground-nut oil (refined)	50	0	24	20	2.43	>0.05
soya-bean oil (refined)	60	0	0	3.3	14.51	< .001
cotton seed oil (crude)	20	19	6.5	10	3.54	0.05

+ Results adjusted for control mortality and sterility

* Using Yate's correction in 2 x 2 contingency table.

Table 30. The sterilising effect on male <u>Diparopsis</u> when exposed to overnight contact with filter paper saturated with graduated aqueous solutions of tepa and subsequently mated with 1 day old virgin females.

% conc. of tepa soln.	No. of males treated	Mating as percentage of control mating	Eggs + laid	% sterility *
0.025	20	70	142	29.5
0.05	20	70	188	80.1
0.10	20	160	275	84.7
0.20	44	109	67	100
0.50	18	0	0	-
1.00	14	42	106	100

* Corrected for control sterility by Abbott's formula.

+ 2 day oviposition period.

Males were completely and consistently sterilised at a tepa concentration of 0.2%. Males sterilised in this way and repetitively mated were shown to be sexually vigorous, in that no significant difference in the mating frequency of treated insects compared with untreated insects occurred ($X^2 = 3.89$). (Table 31)

Table 31. Repetitive mating test of male ꟾꞮꝑꜻꝛꝏꝑꞩꞮꞩ sterilised by overnight contact on

filter paper saturated with 0.2% tepa-water.

Treatment	No. males treated	Mean male mortality (days)	Mean no. of matings per male	Mating frequencies				Total eggs laid	No. sterile	% sterility
				X0	X1	X2	X3			
0.2% tepa water	32	5.2	0.69	16	12	2	2	1346	1313	97.6
water only	10	6.2	1.20	4	2	2	2	645	32	5

X^2 value for mating frequencies 3.89, indicating no evidence of significant

heterogeneity at the 5% level (see also repetitive mating tests in Part I).

2. Marker dye experiments.

The organs most conspicuously dyed were the crop, rectum and ductus simplex of the reproductive system. The distribution of marker dyes between these organs is shown in Table 32. The dye fast-green FCF initially stained the thin-walled crop an intense blue colour, while the rectum, normally orange-brown in colour was stained bright green. After 5 days, the crop was no longer coloured although the rectum was still well stained. Apart from the narrow alimentary canal which is not conspicuous on cursory examination in Diparopsis, no other internal organ was stained. Of the other dyes examined, rhodamine B at a concentration of 0.5% was preferentially absorbed by the ductus simplex of the reproductive system and particularly the segment adjacent to the chitinous simplex as described by Outram & Campion (1967). This suggested a method for staining the spermatophores in future competitive mating experiments, since the precursors of the spermatophore corpus were those bodies which were most intensely stained. This was confirmed by subsequent mating experiments, although after the first mating much of the dye was lost. Methylene blue dyed crop, rectum and ductus simplex but was also toxic, while eosin and nile blue only faintly stained the internal organs. It was concluded that fast-green F.C.F. was the most satisfactory of the dyes tested and was used in all the subsequent bait station experiments.

Table 32. The distribution and persistence of potential marker dyes in crop, rectum and ductus

simplex of male _Diparopsis_ moths following probing on aqueous solutions.

Dye	Conc. %	Time post-treatment (days)	Crop	Rectum	D.simplex	Colour and remarks
Fast Green FCF	0.5	1	+++++	+++++	-----	Crop dyed a bright blue and rectum
		3	+----	+++++	-----	blue-green.
		5	-----	+++++	-----	
Fast Green FCF	0.1	1	+----	+-+++	-----	As above.
		3	-----	+++++	--÷--	
		5	-----	+++++	-----	
Eosin, water soluble, yellow shade	0.1	1	-----	-----	-----	No stain detected.
		3	-----	--÷--	-----	
		5	-÷---	-----	-----	
Rhodamine B	0.5	1	---+	---+-	+++++	Crop dyed red, rectum dyed red-
		3	-----	+--+-	++++	purple, simplex dyed pink (yellow
		5	-----	-----	+--++	under U.V.).
Rhodamine B	0.1	1	-----	-+++++	---+-	As above.
		3	-----	--+++	-----	
		5	-----	----+	----+	
Methylene blue	0.5	1	++++-	++++-	++---	Crop dyed blue, rectum dark green, d.
		3	all dead & not examined			simplex stained differently compared with Rhodamine B.
Methylene blue	0.1	1	----tr	---trtr	-----	V. slight blue colour where
		3	-----	tr tr---	-----	indicated.
Nile blue	0.5	1	trtr---	trtr--tr	-----	Rectum dyed light blue, also anterior
		3	--tr--	+----	+-	part of d. simplex.

+ = intensely coloured
tr = slightly coloured
- = no dye present

3. Bait station experiments.

A series of experiments were first undertaken to determine the effectiveness of virgin females in attracting males to a bait station (illustrated in Fig. 11) under the particular laboratory conditions. The results shown in Table 33 indicated that with each extra female in the bait station an ever increasing number of males were attracted. In the absence of females only a very small number of females approached the station. The results shown in Table 34 indicated that 3 females in the lure holder remained attractive for at least 8 days, although after 6 days some slight loss of attractancy did occur.

A Baffle
B Lure holder
C Filter paper funnel
D Funnel-holder
E Reservoir containing
   chemosterilant solution
F Wick
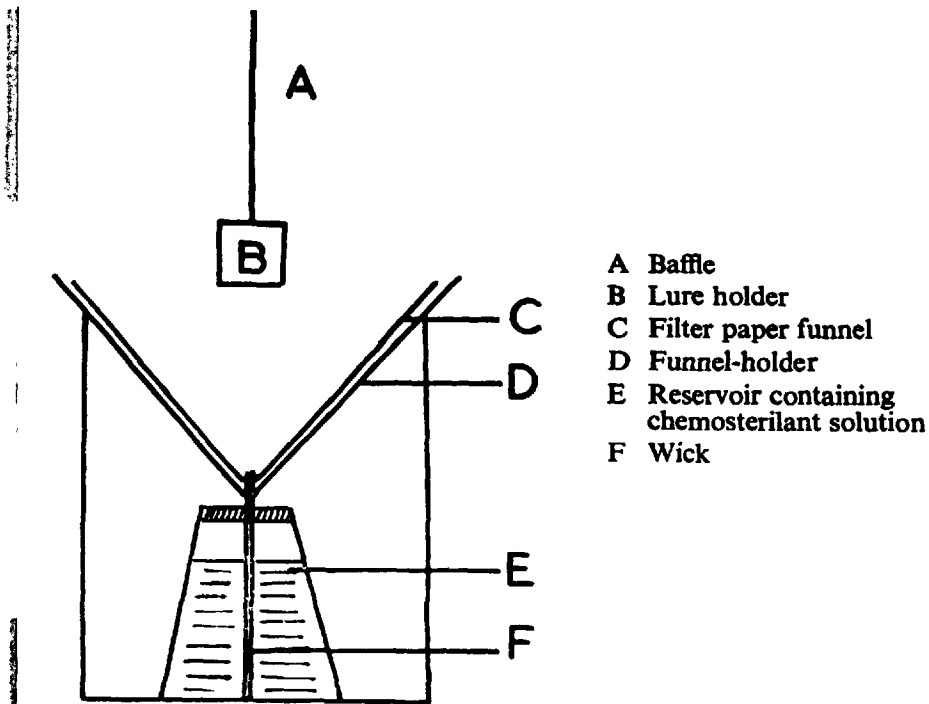
Figure 11 Diagrammatic representation of chemosterilant bait-station.

The results in Table 35 showed the attraction of the same virgin females of increasing age on successive nights to males of constant age. Since the males were 7 days old at the time of release, nearly all those visiting the station actively probed. The results presented in Table 36 showed that when males were released of increasing age on successive nights an increasing number of those visiting the bait station actively probed. Thus 12.5% probed when 1 day old at the time of release, whereas 92.3% probed at the age of 4 days. The males used in those experiments were maintained under constant light before release, in which condition they remain inactive.

Table 33. The effect of an increasing number of virgin females in the bait station on the number of Diparopsis male moths attracted.

No. of females as bait	No. of * replicates	% mean rate of attraction	95% confidence limits
0	3	7.6	5.4-9.8
1	4	24.4	11.4-37.4
2	4	35.2	27.6-42.8
3	13	49.7	40.7-58.7

* 18-40 moths in each replicate.

Table 34. The effect of age of female Diparopsis on the number
of male moths attracted to the bait station using
3 females as lure.

Age of females in days	No. of males released (3-5 days old)	No. of males marked	% rate of attraction
1	42	19	45.2
2	28	13	46.4
3	28	12	42.9
4	31	15	48.4
5	31	13	41.9
6	26	15	57.7
7	33	8	24.2
8	24	8	33.3

Table 35. Attraction of male _Diparopsis_ moths of constant age to a bait station using virgin females as bait and the rate of probing of old males visiting the station.

Age of females as bait (days)	No. of females as bait	Age of males when released (days)	No. of males released	No. of males marked externally (visiting)	No. of males marked internally (probing)	% males visiting bait station	% males probing	% probing of males visiting bait station
1	3	7	13	7	6	53.7	46.2	85.7
2	3	7	11	2	2	18.2	18.1	100
3	3	7	13	5	5	38.5	38.5	100
4	3	7	20	11	9	55.0	45.0	81.8
6	3	7	26	15	11	57.7	42.0	73.3
7	3	7	33	8	7	24.2	21.2	87.5
Totals			116	48	40	41.4	31.5	83.3

Table 36. Attraction of male _Diparopsis_ moths to a bait station using virgin females as lure and the relationship between rate of visitation and rate of probing with increasing age of the males at the time of release.

Age of females as bait (days)	No. of females as bait	Age of males when released (days)	No. of males released	No marked externally (visiting)	No. marked internally (probing)	% males visiting station	% males probing	% probing of males visiting station	Mean % probing of males visiting station
(a) males maintained under constant light until time of release									
1	3	1	43	16	2	37.2	4.7	12.5	
2	3	2	26	11	3	42.3	11.5	27.3	
3	3	3	22	15	11	68.2	50.0	73.3	51.8
4	3	7	17	13	12	76.5	70.6	92.3	
-	0	8	18	1	1	5.6	5.6	100	
(b) males kept in 12 hour dark-12 hour light until time of release									
1	3	1	38	27	3	71.1	7.9	11.1	
2	3	2	46	22	5	47.8	11.9	22.7	
3	3	3	18	10	7	55.9	38.9	70.0	33.8
4	2	4	30	7	6	23.3	20.0	85.7	
-	0	5	28	2	2	7.1	7.1	100	

When the moths were maintained under a 12h. photoperiod throughout
the whole experiment, the rate of probing increase with age
was almost the same as in the previous experiment, even though
considerable flight activity must have occurred. This
confirmed the earlier observation that flight activity in itself
was not an important factor in inducing probing.

4. Response of tepa treated males to virgin
   females in a bait station.

The effect of tepa on the responsiveness of male moths to
virgin females is shown in Table 37. Although treatment
resulted in increased mortality, the rate of attraction was
the same as for the untreated moths. The frequency of treated
and untreated males visiting the bait station indicated no
significant heterogeneity ($X^2 = .044$).

5. Population models.

The results of the computations for the models is shown in
Table 38. By comparing the cumulative mating rates in the
case of no treatment, with the various theoretical treatments,
percentage control is expressed as a percentage reduction in
total mating. The results are summarised in Fig. 12 by plotting
the probit of the percentage control against the log of the
ratio of caged virgin females to wild virgin females. It is
seen that the improvement in control by sterilising attracted

Table 37. Relative rate of attraction of _Diparopsis_ males sterilised by overnight contact on filter paper saturated with 0.2% aqueous tepa compared with males exposed to water alone.

Days post treatment	No. treated males	No. untreated males	No.dead after release		% mortality		No.caught in bait station		% visiting station		Ratio of treated/ untreated males visiting stn.	Age of females (days)	Mean age of males (days) when released
			A	B	A	B	A	B	A	B			
1	57	52	8	0	14.0	0	11	15	19.3	28.8	1:0.67	2	4.3
2	56	62	27	1	48.2	1.6	18	9	32.1	14.5	1:2.21	3	5.3
3	64	58	14	1	21.9	1.7	16	19	25.0	32.8	1:0.76	4	5.0
4	58	60	24	5	41.4	8.3	13	14	22.4	23.3	1:0.96	5	6.0
5	11	29	3	0	26.9	1.1	1	11	9.1	37.9	1:0.24	6	6.0
Totals	246	261	76	7	30.9	2.7	59	68	24.0	26.1	1:0.92		

A = males treated   B = males untreated

Frequency of visitations of treated males to the bait station compared with untreated males (x^2 = 0.044) gave no evidence for significant heterogeneity.

Table 38. Theoretical control of mating by exposing an emerging population (15 males & 15 females) to varying ratios of caged virgin females and by killing or sterilising the attracted males.

Day	No treatment A (Fi)	No treatment B (Ci)	Kill 25:1 A (Fi)	Kill 25:1 B (Ci)	Kill 6.6:1 A (Fi)	Kill 6.6:1 B (Ci)	Kill 3.3:1 A (Fi)	Kill 3.3:1 B (Ci)	Ster 25:1 A (Fi)	Ster 25:1 B (Ci)	Ster 6.6:1 A (Fi)	Ster 6.6:1 B (Ci)	Ster 3.3:1 A (Fi)	Ster 3.3:1 B (Ci)
			Killing attracted males — Ratio of caged to daily emergence of wild virgin females						Sterilising attracted males — Ratio of caged to daily emergence of wild virgin females					
1	15	15	1	1	2	2	3	3	1	1	2	2	4	4
2	15	26	1	1	3	5	6	8	1	1	4	5	6	8
3	15	35	1	2	4	8	7	13	1	2	5	9	7	13
4	15	41	1	4	5	10	8	18	1	3	5	11	10	20
5	15	46	2	5	5	13	8	21	2	4	5	13	8	23
6	15	48	2	5	5	15	8	25	2	5	5	15	7	24
7	15	51	2	6	6	17	9	27	2	5	4	15	6	24
8	15	53	2	7	6	18	9	29	2	5	4	15	6	24
9	15	55	2	7	6	19	9	30	2	6	4	15	5	23
10	15	56	2	7	6	20	9	31	2	6	4	15	5	22
11	15	57	2	7	6	21	9	32	2	6	4	15	5	22
12	15	58	2	8	6	21	9	33	2	6	4	15	5	21
13	15	59	2	8	6	22	9	33	2	6	4	15	5	20
14	15	59	2	8	6	22	9	34	2	6	4	15	5	20
15	15	59	2	8	6	22	9	34	2	6	4	15	5	19
16	15	59	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	5	19
17	15	59											↓	↓
18	15	59	↓		↓	↓	↓	↓	↓	↓				
19	15	59		↓							↓			
20	15	59							↓			↓	↓	↓
↓	↓	↓												
120														
Totals	6913	1800	916	236	2545	702	3941	1057	698	236	1765	482	2293	614
% control as reduction in mating			86.7		64.2		43.0		89.9		74.5		66.8	

A = daily mating rate   B = cumulative mating rate

males compared to killing them is slight at a 90% control level and above, but progressively increases at lower control levels.
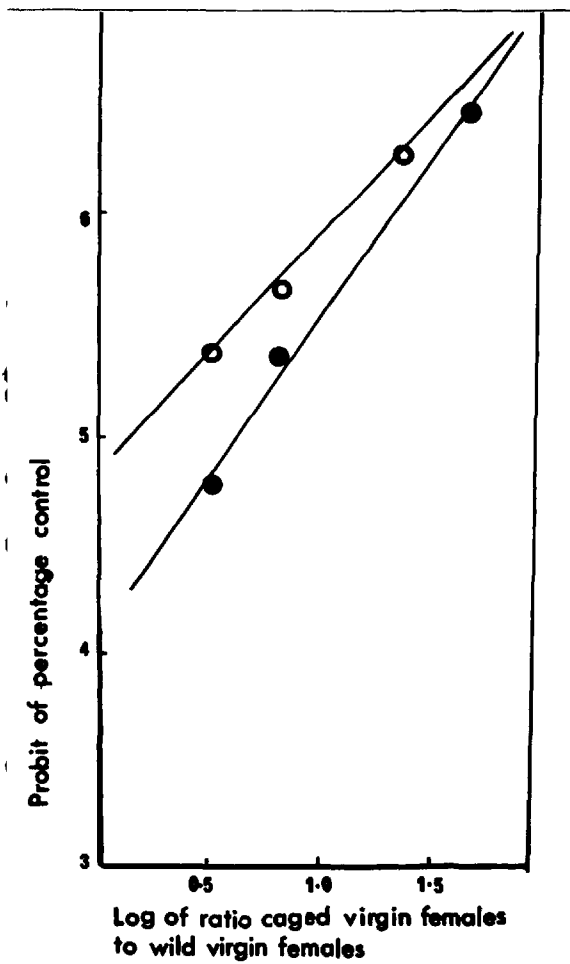
Fig. 12. Relationship between predicted level of control and the ratio of caged to wild virgin females when the attracted males are either killed or sterilised. Solid circles = killing attracted males. Hollow circles = sterilising attracted males.

DISCUSSION

Norris (1934) in reviewing the feeding habits of
Lepidoptera Heteroneura, categorised them into 4 groups.
(1) species which require neither sugar nor water and cannot
take them owing to a reduction of mouthparts, (2) species which
can take sugar and water (i.e. with normal mouth-parts) but
have no necessity for it, (3) species which require water but
not sugar and (4) species which require both sugar and water.

An absolute dependence on water is probably related to
the number of mature eggs and sperm available at the time of
adult emergence. Both sexes of Diparopsis are sexually mature
on emergence and a high level of mating at this time has been
observed (Campion & Outram, 1967).

Another noctuid, the cabbage looper moth Trichoplusia ni
is much more dependent on a water sugar diet and only mates to
an appreciable extent 2 nights after emergence at which time
mature eggs are first found in the common oviduct (Henneberry &
Kishaba, 1967; Shorey, Andres & Hale Jr, 1962). A similar
situation was reported by Ouye et al (1964) for the pink boll worm
Pectinophora gossypiella. The fall armyworm moth Spodoptera
frugiperda an active feeder usually mates the following night
after emergence (Young et al, 1968). Another noctuid moth
Heliothis zea is sexually mature on the night of emergence but
according to Callahan (1961) the rate of feeding is initially

determined by the capacity of the crop. When the abdomen of the moth is packed with fat body and/or eggs the crop is compressed and no feeding response is obtained. With diminished fat body in the males and oviposition in the females, the crop is able to expand and feeding occurs.

Typical metereological data from cotton growing areas of Central Africa where Diparopsis is prevalent were reported by Tunstall, Sweeney and Rose (1958). For the 1956/7 cotton growing season at Gatooma, Rhodesia, the mean temperature was 22.5°C ranging from 20.8-32.6°C and the mean R.H. was 70% ranging from 54.5-78%. In Makanga, Malawi, the mean temperature was 28.5°C ranging from 22.5-33°C and the mean R.H. was 78.2% ranging from 63-85%. Under such climatic conditions the rate of probing by male Diparopsis moths is therefore not likely to be greatly increased from the rate found in the laboratory experiments.

Experiments performed at ambient Rhodesian dry season conditions where humidity was very low suggested that some increase in the probing rate occurred by using 10% D-glucose compared with using water (Campion & Outram, 1967). No such increase occurred however when the moths were maintained at 70% R.H. prior to exposure to the sugar solution while substitution of water by 10% cane sugar or 1% honey water made no difference to moth longevity. The combination of tepa and sugar in aqueous solution may anyway be undesirable since the fermentation of

the sugar would result in an acidic medium known to cause a rapid
degradation of the chemosterilant (Beroza & Borkovec, 1964).

By use of the marker dye technique the effectiveness of
virgin females in attracting the male moths to a bait station
was clearly shown. However even under these conditions the
moths were at least 3 days old before a high proportion of those
visiting the station actively probed. The life expectancy
of the male moth at 27°C and 70% R.H. is between 7-9 days
by which time the fat body is exhausted. It is therefore
concluded that a method of control for Diparopsis based on
autosterilisation by probing is unlikely to be successful since
several matings could occur before the probability of probing
was very great. Similar problems were noted by Young
et al (1969) who developed a chemosterilising bait station
for the fall armyworm Spodoptera frugiperda. Although no
direct observations on feeding behaviour were made, they
concluded that the incomplete sterility achieved was related
to the lack of feeding response, particularly on the night of
emergence. Honey or sugar water are claimed to be necessary
for ovarian maturation in adult Autographa (Vojnits, 1969).
Although no observations were made on the food requirements
of the male moth in the present study, an insect with such
a feeding habit might be more suitable for the autosterilsation
method.

The possibility of using an alternative diluent to water to facilitate direct penetration of the chemosterilant following momentary contact was considered. The natural vegetable oils selected for this purpose however, when applied to the male moth were generally non-toxic but all reduced mating effectiveness and were therefore unsuitable. Barlow & Turner (1967) showed that tepa would not persist on a non-porous surface because of its high volatility. For this reason the contact sterility effect of surface films of the chemosterilant or glass or similar substances were not considered.

Male moths could be consistantly sterilised by overnight contact on filter paper saturated with 0.2% aqueous tepa. The repetitive mating test showed that the moths treated in this way remained competitive, although some reduction in longevity occurred.

Henneberry et al · (1966) reported that following ingestion of tepa, the cabbage looper moth Trichoplusia ni became markedly less responsive to the female sex attractant. Diparopsis males however sterilised by overnight contact on filter paper saturated with aqueous tepa and where ingestion must have occurred remained equally attractive. The development of an autosterilisation control method based on the overnight contact of males on a tepa treated surface attracted by virgin females or eventually the synthesised pheromone, may therefore be possible. The immediate difficulty to be anticipated is

the dispersal of the adults after treatment.

From the population models based on using virgin females
as lure, the advantages of sterilising attracted males as
opposed to killing them, at least at the 90% control level
and above is not all that great. Lures   that would attract both
sexes of moth however are expected to be more effective than
sex-specific attractants (Knipling & McGuire, 1966). The
further development of light traps at present being considered,
for example for the control of the tobacco worm (Gentry et al
1967) may be one such system, while the combination of sex
attractant and light trap referred to in the introduction
might also be a possibility for future investigation. However
all there methods require the solution to the basic problem
of how to effectively treat the insect once it has been caught
and then to return it to the environment with minimal handling.
This problem has always to be related to the slender resources
available in the under-developed countries where this
particular cotton pest is prevalent.

The other possibility exists of chemosterilising either
mass-reared or even mass-collected insects. Such a programme
is at present being conducted in the United States against
codling moth Carpocapsa pomonella (Butt, 1968).

Tepa applied topically to male Diparopsis effectively
induced sterility and the moths were sexually competitive
under laboratory conditions (Part I). The natural population

would however have to be first reduced to a very low level
by conventional methods to avoid the release of enormous
numbers of insect. Moreover since only males are effectively
sterilised by tepa, the insects would have to be sexed and
only males released. Further elucidation of these problems
must await future field evaluation.

PART III.

The persistance of chemosterilants when applied to insects.

INTRODUCTION AND REVIEW OF LITERATURE

In part II reference was made to the toxicity, mutagenicity and even phytotoxicity of tepa and related aziridine chemosterilants. It was nevertheless suggested that under certain circumstances tepa might possibly be used either for the sterilisation of mass reared and mass collected insects or alternatively in an autosterilising bait station. Moreover as Borkovec (1968) pointed out the potential hazards involved in each particular instance would first have to be carefully delineated.

Tepa in Mexican fruit flies Anastrepha ludens for example was determined colorimetrically by Chang & Borkovec (1966) and radiometrically in houseflies by Chang et al (1966). 90-95% of the tepa residue was lost within 72 hours. Plapp et al (1962) reported that metepa degradation determined radiometrically was completed within 24 hours in adult mosquitoes C. tarsalis and 48 hours in adult houseflies M. domestica. Using gas chromatographic methods of analysis Morgan et al (1968) found that after oral uptake of metepa in houseflies, 90% had degraded in 72 hours in female flies and 99% in male flies. Chamberlain & Hamilton (1964) found that while the screw worm fly Calligotroga hominivorax excreted P^{32} labelled metepa twice as fast as the stable fly Stomoxys calcitrans, only 18.4% from males and 21.3% from females was excreted within 24 hours. Ladd et al (1968) concluded that following treatment

of adult Japanese beetle <u>Popillia japonica</u> by immersion in aqueous tepa solutions, very little chemosterilant remained after 48 hours when measured colorimetrically, by which time no water soluble residues remained in the outer layer of the cuticle.

Maitlen & McDonough (1967) determined the persistence of tepa by the colorimetric method when applied by aerosol application to codling moth <u>Carpocapsa pomonella</u> and found that between 88-97% degradation occurred within 72 hours after treatment. Cox <u>et al</u> (1967) determined the persistence of orally applied tepa in the fall armyworm moth <u>Spodoptera frugiperda</u> by gas chromatographic and radiometric procedures. The radiometric analyses showed considerably more tepa than did the specific GLC analyses, indicating that C^{14} labelled fragments of the tepa molecule were being analysed as tepa. Within 24 hours more than 90% of the tepa had been degraded from moths ingesting as much as 100 µg per moth, while more than 95% had disappeared within 48 hours.

The distribution of tepa to various parts of the body and internal organs after topical application to the boll-weevil <u>Anthonomus grandis</u> was studied radiometrically by Hedin <u>et al</u> (1967a). The chemosterilant was absorbed almost immediately into the haemocoel reaching a peak in 1-3 hours. The trend paralleled a decline of tepa on the cuticle to within 10%

within 6 hours. Values on the differential distribution ratio for body parts and organs were determined by injection and topical treatment of abdomen. Selective concentration occurred in the foregut, testes and wings with injection and wings only with topical treatment.

It would therefore seem that a fairly rapid degradation of tepa would be expected in Diparopsis. Previous workers had not however related the speed of breakdown to temperature after different methods of application. This was the aim of the present series of experiments in order to be able to predict the environmental contamination at temperatures prevailing in areas where Diparopsis was prevalent.

In the absence of a gas chromatograph equipped with a flame photometric detector which was used by Bowman & Beroza (1966) for the detection of trace amounts of tepa, metepa, methiotepa, hempa and apholate, a colorimetric method of analysis was adopted.

MATERIALS AND METHODS

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 of 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 x 6 x $3\frac{1}{2}$ in). Before extraction dead moths were discarded and samples of twenty-five moths were homogenised for 5 minutes in a high speed homogeniser in 50 ml of chloroform. To determine the amounts of tepa in the cuticular layer, whole moths were shaken in chloroform for 5 minutes. 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 (1955). 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 pH4 buffer and 1 ml of the χ-(4-nitrobenzyl)
pyridine reagent. The mixtures were heated for 20 minutes in a
boiling water bath and then cooled in an ice water bath. To
each tube was added in quick succession 4 ml of acetone, 1 ml
of M. aqueous potassium carbonate and distilled water to make
the volume up to 10 ml. To avoid turbidity Hyflo Super-cel
was added and after filtration the colour intensitiy 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 minutes. The
residue values obtained were corrected for the apparent residues
found in control samples. Each sample was assayed at two
dilutions and the mean value recorded.

RESULTS

The results showing the varying rate of tepa degradation
at 15, 20 and 27°C after injection and topical application,
6aking the mean of the three replicates are given in Table 39.
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. 13 & example in
Appendix 3). From the regression equations (Table 40) 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 hours at
27°C to 18.6 hours at 15°C. After topical application, tepa
decomposition occurred much more slowly reaching the 50% level
after 45.6 hours at 27°C, 72.3 hours at 20°C and 143.3 hours
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 hours. The rate of decomposition of tepa
applied to topically treated insects was lower than the rate
of absorption (Fig. 14).

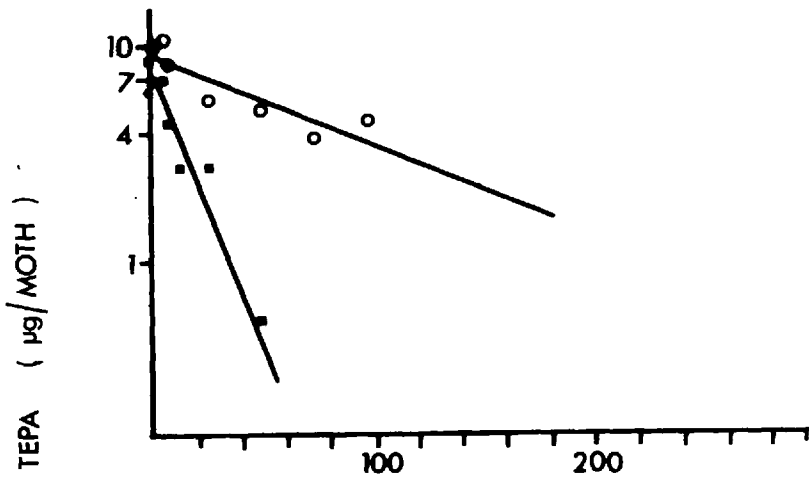Fig. 13. To show the rate of tepa degradation inmmale <u>Diparopsis</u> moths

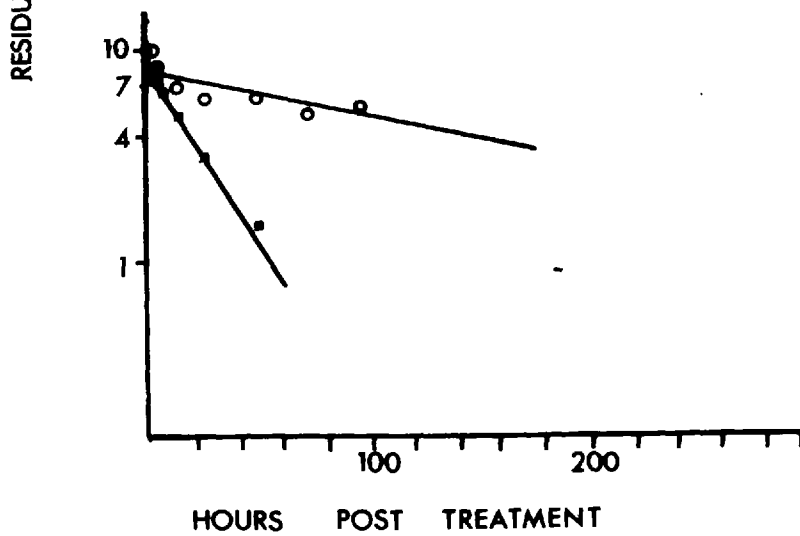after injection and topical application at A, 27°C; B, 20°C

and C, 15°C.

▌ = injection application, o = topical application.

RESIDUAL TEPA (μg/MOTH)
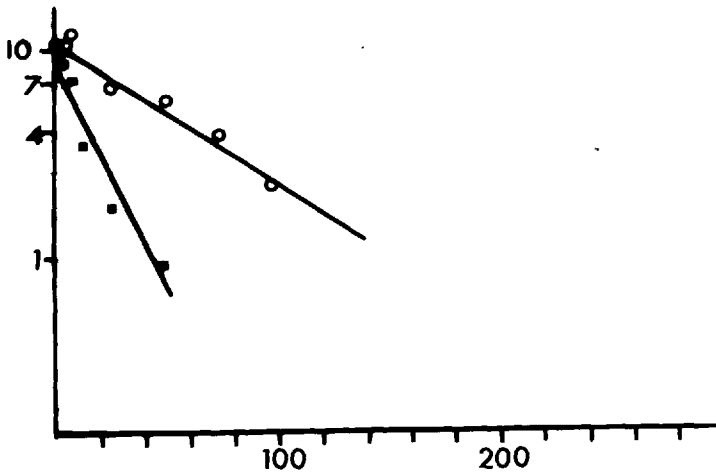
HOURS POST TREATMENT

Table 39. Residues of tepa in µg (mean of 3 replicates).

(A) Extraction of homogenised insects

(B) From surface washes of whole moths after topical application.

Interval post-treatment (hours)	Topical Application			Injection application			20°C
	15°C	20°C	27°C	15°C	20°C	27°C	
0	10.7	11.0	10.4	8.5	9.2	9.4	8.5
3	8.6	11.3	10.6	7.5	7.3	8.2	-
6	7.6	8.2	10.9	6.5	4.4	6.9	8.0
12	7.2	8.0	8.1	5.0	2.7	3.4	-
24	7.4	5.7	6.7	3.0	2.8	1.7	5.6
48	6.1	5.2	5.7	1.3	0.6	1.0	4.0
72	6.0	3.8	3.9				2.6
96	5.5	4.4	2.3				2.4

Table 40. Tepa degradation in male **Diparopsis.** Summary table to show evidence for linearity of regression lines; regression equations and 50% breakdown time.

| Temperature °C	Method of treatment	Method of extraction	Mean squares		F ratio	Regression equation	50% breakdown time (hours)
			regression variance	within dose variance			
15	injection	homogen- ization	0.0013	0.0079	0.16	y=0.923-0.016x	18.6
20	"	"	0.0540	0.0221	2.44	y=0.892-0.026x	19.3
27	"	"	0.0124	0.0323	0.28	y=0.904-0.021x	14.3
15	topical	"	0.0120	0.0214	0.48	y=0.843-0.0021x	143.3
20	"	surface wash of whole insects	0.0056	0.0251	4.64	y=0.957-0.0043x	72.3
27	"		0.0077	0.0660	0.90	y=1.002-0.0066x	45.6
20	"		0.0050	0.0044	1.12	y=0.9121-0.0064x	48.6
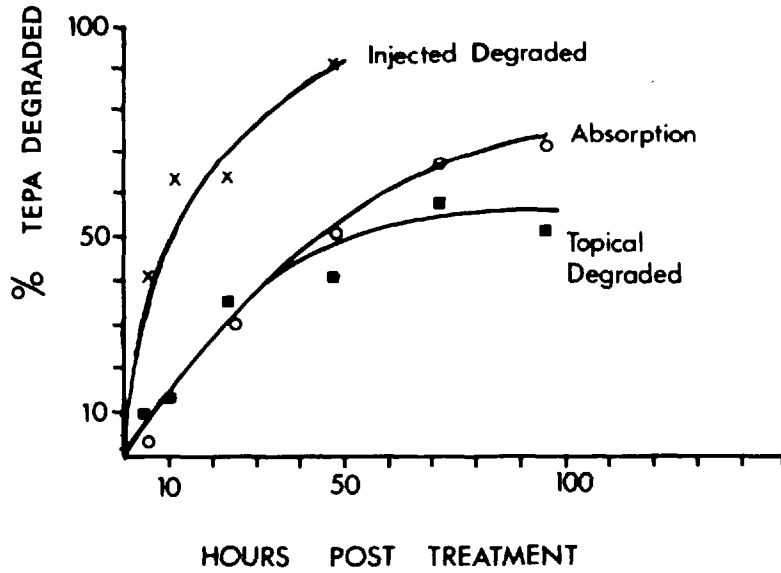
Fig. 14. To show the varying rate of tepa degradation at 20°C
when applied by injection, topically or absorbed
through the cuticle.

DISCUSSION

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 metabolised in 19.3 hours when injected, but in 72.3 hours when applied topically. It is evident from Fig. 14 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_ (see part IV). It may be concluded that if male moths are sterilised 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 (part II) 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 Q_{10} of 1.3 for injected treatments). Typical meteorological data from the cotton growing areas of Central Africa where Diparopsis is prevalent were reported in part II. The mean temperature in Makanga, Malawi, during the 1956/7 cotton growing season, for example, was 28.5°C; in Gatooma, 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 sterilised 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 sterilised 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 the use of oily solvents which, by diffusing over the whole insect may promote faster penetration of the sterilant (cf. Lewis, 1963). 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.

PART IV.

The effect of chemosterilants on the reproductive-organs,

sperm and embryogeny of insects.

REVIEW OF LITERATURE

1. Gross effects of chemosterilants on the
    insect reproductive system.

Observations on the effects of alkylating agents against
the insect reproductive system have been mostly confined to
dipterous insects. The effects on ovarian development are
usually quite dramatic. Treatment of the housefly Musca domestica
with tepa, thiotepa and apholate was followed after an interval
of 1-3 days by such effects as condensation and pyknosis of
nuclei, vacuolisation of cytoplasm and a general atrophy of
the follicular epithelium. (Morgan & LaBreque, 1962, 1964;
Landa & Řežábová, 1965; Combiesco et al, 1967). Matolín (1969)
found that the effect varied from complete inhibition of the
development of ovaries, reduction in oviposition, to the
formation of normally developing eggs; while Kissam et al (1967)
reported little change in ovarian development following
application of tretamine and methylmethane sulphonate.
Chamberlain & Barret (1968) studied the effect of apholate on
ovarian tissue in the stable fly Stomoxys calcitrans (L) and
found that the chemosterilant interfered with the incorporation
of tritiated thymidine into the DNA of some of the nuclei
of nurse cells and follicular cells, when treatment occurred
shortly after adult emergence. In older insects the main effect

was the induction of dominant lethals. This probably explains the variable results obtained by other workers when the age of the insect at the time of treatment was not exactly known. Similarly Crystal & LaChance (1963) and LaChance & Crystal (1963) showed that the effects of several alkylating agents on the ovaries of the screw worm fly, Cochliomyia hominivorax (Coquerel) varied according to the age of the insect at the time of treatment. When treated at the age of 0-4 hours, oogenesis was inhibited, while at the age of 24 hours mutations were induced. Subsequently LaChance & Leverich (1968) found that treatment completely disrupted the endomitotic replication in the nurse cells. Reduction in the size of the ovaries accompanied by signs of degeneration and chromatin clumping was also noted following apholate treatment of Drosophila melanogaster Meigen (Cantwell & Henneberry, 1963), the eye-gnat Hippelates pusio Loew (Schwartz, 1965) and the mosquitoes Culex pipiens quinquefasciatus Say (Murray & Bickley, 1964) and Aedes aegypti (L) (Rai, 1964) and the parasitic wasp Bracon hebetor Say (Valcovic & Grosch, 1968). Smittle et al (1965) treated the German cockroach Blatella germanica L. with tepa and found that the basal oocytes were smaller in number and the usual disintegration of the ovaries gradually occurred.

In contrast, the effects of aziridine chemosterilants on the male reproductive system are generally not so marked. Cantwell & Henneberry (1963) observed that male Drosophila fed on a diet containing 1% apholate, ceased producing sperm

and eventually developed a general necrosis of the germinal
epithelium. A similar effect, together with a general
reduction in the size of the testes was observed by Schwartz
(1965) when <u>Hippelates</u> fed on 1% metepa and tepa contained in
sugar water. Cline (1968) applied 0.3% thiotepa to the diet
of houseflies. After 4 days on the treated diet, whole
mounts of the testes stained by the feulgen method indicated
that a marked reduction in spermatogonia had occurred as a
result of treatment, although the structure of the testes
as a whole remained intact. Kissam <u>et al</u> (1967) however after
treating houseflies with tretamine and methylmethane sulphonate,
by incorporating them into the diet of the adult insect
failed to detect any histological differences as a result of
treatment.

Hedin <u>et al</u> (1967b) showed that treatment of 1 day old
male boll-weevils <u>Anthonomus grandis</u> Boheman by tepa injection
caused a significant reduction in the size of the testes when
measured 14 days after treatment. A similar effect was
observed by Reinecke <u>et al</u> 1969) after oral application of
several chemosterilants including apholate and busulphan.
Testes size was also reduced in the alfalfa weevil <u>Hypera postica</u>
(Gyllenhal) following apholate treatment by topical application
in acetone (Sprenkel & Yendol, 1967). On the other hand
Ezueh & Hoopingarner (1967) working with the cereal leaf-beetle
<u>Oulema melanopus</u> L. found no detectable effect on the testes

when the adults were treated by immersion in apholate solutions causing complete sterility. Nakayama & Nagasawa (1966) failed to detect damage to the testes of the Azuki bean weevil Callosobruchus chinensis L. following treatment with sterilising doses of metepa, and Hamilton & Sutter (1969) found no effect on the testes of the Southern corn rootworm Diabrotica undecimpunctata howardi Barber by oral application of apholate, although a reduction in accessory gland secretion was claimed.

The extent of observable damage to gonadal tissues after chemosterilant treatment of the adult insect is clearly related to the extent of spermatogenesis or oogenesis occurring at the time of treatment and is therefore particularly noticeable in long lived insects such as beetles where active gametogenesis occurs in the adult stage.

Damage to the mid gut epithelium of the boll-weevil was also caused by chemosterilant treatment after oral application, a phenomenon also noted after radiation treatment (Reinecke et al, 1969; Riemann & Flint, 1967). As a result of such damage, digestion was affected leading to early mortality and a lack of competitiveness.

In many Lepidoptera both mature sperm and ova are formed either just before or soon after adult emergence. The process of spermatogenesis has been described in detail for the rice stem borer Chilo suppressalis Wlk.(Kurihara, 1929), the silkworm Bombyx mori L. (Sado, 1961), the sugarcane borer Diatraea

saccharalis F. (Virrki, 1963) and tho European corn borer
Ostrinca nubilalis Hubner(Chaudhury &Raum, 1966). Primary
and secondary spermatocytes were found in the larval stage
and spermatids reached peak numbers in early pupal life, while
virtually only fully formed spermatozoa were found in the adult
stage. Excessive damage to the testes would therefore not be
expected as a result of tepa treatment of such insects at the
adult stage, since the period of active cell division has
already been completed.

2. The effect on egg development when female insects
are mated to chemosterilised males.

(a) Dominant lethality. The most frequent form of sterility
induced in insects by chemosterilant treatment has been the
induction of dominant lethality, although other effects
related to sperm inactivation or endocrine misfunction may
occur. Dominant lethal mutations as a result of the action of
radiation were first observed by Hertwig (1911) following
the irradiation of amphibian sperm. Subsequent work by
Muller (1927) showed that irradiation of insect reproductive
cells did not generally hinder the maturation of the treated
cell into a gamete but prevented the subsequent zygote from
developing to maturity. Such lethals are now known to be the
result of major chromosomal structural changes, since insects
with small chromosomal deficiencies are usually viable, normally

as recessive genes. Variation in sensitivity to X-rays to the induction of dominant lethals has been observed in different strains of <u>Drosophila</u> while as in the case of sterility induced by chemosterilants, the age of the male and the stage of spermatogenesis at the time of radiation are also important (Fahmy &Fahmy, 1954). A lethal mutation may be expressed at any time during the insect's life, but death usually occurs well before hatching. Embryonic death is associated with a depression in the mitotic rate of the embryo with a complete cessation of mitosis often occurring after the second or third cleavage division. Death is also often accompanied by polyploid cleavage nuclei (Demerec & Fano, 1944) indicating that DNA synthesis may persist for some time after mitotic division has ceased.

Sonnenblick (1940) and Sonnenblick & Henshaw (1941) determined the effect of egg development in <u>Drosophila</u> by treating the parents with X-rays at doses of 2000-5000r. In general both distorted as well as normal kinds of development occurred, and occasionally at an age when embryos were highly differentiated, some consisted of a structureless mass of cells.

The aziridine alkylating agent tretamine injected into male <u>Drosophila</u> consistantly induced dominant lethal mutations. Virtually no embryological development occurred, although

examination of egg samples showed that they had been inseminated. LaChance & Riemann (1964) treated both male and female Cochliomyia with either radiation or tretamine and similarly found in both instances a large number of chromosome aberrations in the first two cleavage divisions. Matolín (1969) however found that treatment of adult houseflies with apholate, tepa or hempa resulted in either normally developing embryos or the occurrance of autolysis after the first few cleavage stages. Treatment with apholate also sometimes resulted in irregularly shaped eggs. Smittle, Schmitt & Burden (1966) investigated the effect of tepa sterilisation of male Blatella, on subsequent embryonic development in eggs. They found that some degree of normal development occurred in 15 out of 21 oothecae; while in 1 ootheca both normal and abnormal development was occurring. Nakayama & Nagasawa (1966) examined eggs from matings between metepa sterilised male Callosobruchus and untreated females and found that development progressed only to an early cleavage stage with no blastoderm formation, while necrosis of the cleavage nuclei and plasmolytic atrophy of the vitellus were observed in eggs 48 and 96 hours after deposition. Flint et al (1968a) observed without cytological examination, that when tobacco budworm Heliothis virescens F. adults were sterilised with several aziridine alkylating agents, no development in the unhatched eggs occurred. A similar observation was made by Solomon (1966) following tepa treatment of male carpenter moths Prionoxystus robiniae Peck.

(b) <u>Reciprocal translocation.</u> If chromosme breakage and loss
of chromosome parts are the basis of dominant lethal mutations
it would be expected that insect species with diffuse centromeres
would prove very resistant to their induction, since all fragments
would be retained in the daughter nuclei. Such a condition
occurs in the Lepidoptera (Federley, 1943; Suomalainen, 1953;
Bauer, 1967; Virrki, 1963). Thus while 5Kr are required to
sterilise male screw worm flies <u>Cochliomyia hominivorax</u>
(Bushland & Hopkins, 1951) and 4Kr the adult Mexican fruit fly
<u>Anastrepha ludens</u> (Rhode <u>et al</u>, 1961), 40Kr are needed to
sterilise the codling moth <u>Carpocapsa pomonella</u> (Proverbs &
Newton, 1963), 30Kr for the European corn borer <u>Ostrinia</u>
<u>nubilalis</u> (Walker & Brindley, 1963), the tobacco budworm
<u>Heliothis virescens</u> (Flint &Kressin, 1968) the cabbage looper
<u>Trichoplusia ni</u> (North &Holt, 1968) and the sugar cane borer
<u>Diatraea saccharalis</u> (Walker & Quintana, 1968).

Broken chromosomes can reunite in many ways. One well
known rearrangement is reciprocal translocation, which is
the rejoining of at least two different broken chromosomes.
Most reciprocal translocations involve no chromosome loss, and
can be transmitted to the offspring. However some chromosomal
rearrangements yield one or two acentric fragments and
chromosomes with two centromeres. At anaphase of somatic
division a dicentric chromosome produces a bridge that when
broken results in daughter cells deficient of some portion
of the genetic maternal. According to LaChance, North & Klassen

(1968) and LaChance, Degrugillier & Leverich (1970), late death
of the embryo is typical of reciprocal translocations in
haploid and possibly also in diploid organisms.

(c) <u>Enzyme inhibition.</u> The production of dominant lethal
mutations by changes other than chromosomal ruptures cannot
be completely ruled out. It was noted earlier that high doses
of radiation were needed to induce complete dominant lethality
in several Lepidoptera compared to much lower doses for
dipterous insects. Such a dose differential does not appear
to exist when chemosterilants are applied. Thus male houseflies
are sterilised (SD_{50}) by injected doses of 0.1 µg tepa, 0.404 µg
apholate and 1.30 µg of metepa (Chang & Borkovec, 1964), for
an insect weighing approximately 15 mg. The values for
<u>Diparopsis</u> males weighing approximately 100 mg are 0.73 µg for
tepa, 1.76 for apholate and 2.81 µg for metepa (Part I) and are
therefore on a weight basis almost the same for tepa and apholate,
while metepa is relatively more active against <u>Diparopsis</u>
than <u>Musca.</u> This certainly suggests a different mode of
action of such chemosterilants compared with gamma-radiation.
Von Borstel (1955) proposed that the disruption of DNA synthesis
might be caused as the result of treatment.

Inhibition or increased activity of enzyme systems has
been widely reported following treatment of many animals
both with radiation and chemical mutagens (Bacq &Alexander, 1955).
Kugler <u>et al</u> (1956) concluded from a study of the reproductive system
of the   female cockroach <u>Periplaneta americana,</u> that there

was a relationship between alkaline   phosphatase activity,
glycogen and nucleic acid synthesis. Moog (1946, 1962)
reviewed the significance of alkaline   phosphatases and
concluded that they were commonly found in the cytoplasm of
growing, regenerating and secretory cells in which protein
synthesis was occurring. There also seemed to be a correlation
in such cells between the content of nucleic acid and
phosphatases.

Alkaline   phosphatases were also often localised in the
reproductive organs of many organisms. Day (1949) however
reported the absence of alkaline phosphatase activity in the
gonads of Blatella, Locusta and Lucilia, although present in
the spermatocytes of Periplaneta. Saxena (1969) similarly
reported the absence of alkaline phosphatase activity in the
gonads of the grasshopper Poecilocerus pictus and the
Periplaneta. According to Mann (1964) processes associated
with spermatocyte development show a progressive decline of
phosphatase activity in the nuclei and a simultaneous disappear-
ance of glycogen. However the phosphatase activity of sperm
towards ATP has been noted by many workers for many animals
including mammals, fish and echinodemus and is believed to be
associated chiefly with the flagellum. One may suspect that
yet again, the variations in phosphatase activity reported
depends on the stage of gametogenesis at the time the observations
were made.

Mendoza & Peters (1968) showed that alkaline phosphatase
was present in the gonads of the Southern corn rootworm
Diabrotica undecimpuncta howardi Barber. They concluded
that inhibition of the enzyme occurred in both testes and
ovaries as the result of injection treatment with apholate,
although the effect was only detected histochemically at
toxic levels of the chemosterilant. Turner & Maheswary (1969)
using a biochemical assay technique   reported high alkaline
phosphatase activity in the developing ovaries of the yellow-
fever mosquito Aedes aegypti. They questioned however the
relationship between sterility and enzyme inhibition, since
by comparing the effect of apholate on resistant and susceptible
strains they found the inhibition of the enzyme to be similar
in both instances, whereas sterility was induced in the
susceptible but not in the resistant strain.

In Lepidoptera where spermatogenesis is generally
completed by the time of adult emergence, phosphatase activity
would be more likely to be associated with the flagella
mitochondria than with nucleic acid synthesis; and therefore
a reduction in such activity would probably lead to a loss
of motility.

(d) Sperm inactivation. If sperm inactivation occurred then
fertilisation of the eggs would not occur giving the appearance
of dominant lethality. Whiting & Von Borstel (1954) reported

that after nitrogen mustard treatments, sperm inactivation occurred at far higher doses than those required to induce dominant lethals in all the Habrobracon sperm, while Grosch & Valcovic (1964) observed that the exposure of Habrobracon males to topical applications of apholate (0.01-0.1%) produced only a minor amount of sperm inactivation at doses giving 40-80% dominant lethals in the sperm. LaChance (1966) however found that tarsal contact treatments of Habrobracon males with tretamine produced virtually no sperm inactivation whereas treatment with tepa produced significant amounts of sperm inactivation even at sub-sterilising doses. Fahmy & Fahmy (1958) showed that the high rate of unhatched eggs from female Drosophila mated to males treated with an amino acid mustard, caused cell damage and did not truly reflect a high incidence of dominant lethal mutations in the sperm; thus eggs deposited by the inseminated females were laid unfertilised. The inhibition of the sperm enzyme systems particularly associated with ATP and hence flagellar activity, might provide such a mechanism. Loss of motility in sperm suspensions is often accompanied by the formation of clumps or aggregates due to agglutination or flocculation of spermatozoa . Mann (1964) summarised the extensive literature on the many ways agglutination can be produced, including significantly perhaps treatment with heavy metal salts and organic spermicidal agents. A method for the

determination of the rate of agglutination if any that occurred in the transmitted sperm of <u>Diparopsis</u> following tepa treatment of the male insects should therefore indicate whether such a phenomenon is an important component of sterility.

The male reproductive system of <u>Diparopsis</u> was described by Outram & Campion (1967). In the present study the female reproductive system was examined and the gross effects of tepa on the reproductive systems of both sexes was determined. The effects on embryological development in the egg following tepa treatment of the male parents was investigated in <u>Diparopsis</u> and <u>Autographa</u>.

By means of histological tests the effect of tepa treatment on the relationships between DNA, glycogen and phosphatase activity in mature spermatozoa was undertaken and related to sperm agglutination in <u>Diparopsis</u>. The ultra-structure of the spermatozoa of <u>Diparopsis</u> was determined and possible damaging effects of tepa likely to cause or contribute to sperm inactivation were sought.

METHODS

1. **Preparation of longitudinal sections
   of whole abdomens of Diparopsis.**

The insects were treated by injection with 10 µg of tepa.
After an interval of 3 days the moths were fixed in boiling
Bouin's fluid and pricked with a tungsten needle to facilitate
penetration of the fixative. Wax blocks were prepared in the
standard manner and sections cut at a thickness of 8 microns.
They were stained with Delafield's haematoxylin and sometimes
counterstained with eosin. Sections from untreated insects
were similarly prepared.

2. **Preparation of sections of testes
   for general observation.**

Moths treated with 10 µg of tepa by injection were
dissected after an interval of 3 days and the testes transferred
to cold Bouin's fluid. After preparation of wax blocks, sections
were cut at a thickness of 3-4 microns and stained with
Delafield's haemotoxylin.

3. **Embryological development of eggs fertilised
   by sperm of chemosterilised males.**

To determine whether extensive embryological development
occurred in the egg after chemosterilant treatment of the parent

insect, male Diparopsis were treated with varying doses of tepa, apholate and metepa and mated to untreated females by the standard method described in Part I. Newly laid eggs are green in colour and during the course of normal development turn grey. Eggs from unmated females remain green throughout this period. In therefore assessing the effect of the chemosterilant on egg development from mated females, counts were made of green eggs which indicated either that no development had occurred or at the most development to only the first few cleavage stages, and grey eggs indicated considerable development although death occurred before hatching. Hatched eggs which occurred in the controls or lower dose levels of chemosterilant were not included in the assessment.

For the histological examination of developing eggs, male Autographa moths were injected with 10 µg tepa, a dose known to cause 99% sterility in eggs subsequently laid by mated females (Part I). Following incubation for 3 days at 27°C and 70% R.H. eggs which had been laid on filter paper were randomly selected and plunged into hot Bouin's fluid. On cooling the eggs were pricked by a fine tungsten needle to facilitate the penetration of the fixative. Wax blocks were prepared using the double embedding method of Peterfi described by Pantin (1964). Sections were cut at a thickness of 6 microns and stained with Delafield's haematoxylin and haemotoxylin and eosin. Sections of eggs from untreated parent insects were

similarly prepared.

4. **Treatment of testicular preparations by the Feulgen reaction, the 'PAS' technique and for phosphatase activity.**

Sections of testes of 4 micron thickness were prepared from insects treated 3 days earlier with 10 μg of tepa by injection, together with sections from untreated insects. The procedures for the Feulgen and PAS staining according to Pearse (1960) were adopted, and the sections from treated and control insects were stained under identical conditions.

For the determination of alkaline phosphatase activity again the procedure according to Pearse (1960) was followed. The testes from treated and untreated insects were dissected out and transferred to acetone previously cooled to $-20^{o}C$ and left overnight in a deep freeze after a further change of acetone. Wax blocks were prepared using the standard vacuum embedding technique and stored in a deep freeze. The standard staining technique was used with incubation times of 4, 8 and 24 hours. A series of sections of testes from treated and untreated insects were stained under identical conditions. Four replicates were carried out and at least two slides from each testes were examined. Blank slides containg sections immersed in boiling water for 5 minutes to inactivate the enzyme were also included.

Differences in staining intensity as a result of treatment
were measured by a Vickers scanning microdensitometer.
20 readings were taken for each relicate test, each from
a different section. The mean densities were calculated and
expressed as a percentage difference. For estimation of the
phosphatase density, readings were taken using a x40 objective
both from the centre and periphery of the sections which
consisted almost entirely of mature spermatozoa.

Feulgen densities were determined for cut cross-sections
of sperm bundles through the head region   using a x100 objective.

The densities of sperm heads stained by the PAS method were
similarly determined.

5. Agglutination effects of tepa on sperm
   of Diparopsis

Spermathecae from mated female Diparopsis were dissected
after one mating night following the standard mating procedure
described in Part I. After fixation in Bouin's fluid, wax
blocks were prepared. Sections were cut at a thickness of
6 microns and stained with Delafield's haematoxylin and eosin.
Observations were made under the low power microscope and the
degree of agglutination categorised as 'severe', 'slight' or
'no effect'.

6. Preparation of sperm.

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 24 hours in phosphate-buffered 2-5% glutaraldehyde, post-fixed for 1-2 hours 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 500A° of gold/palladium and examined in a Cambridge Instruments Stereoscan electron microscope.

EXPERIMENTAL

1. Structure of the reproductive system of Diparopsis.

The male reproductive system of Diparopsis does not differ essentially from other noctuids and was described by Outram & Campion (1967).

The female reproductive system is of the Ditrysian or Diplotreme type, having two separate openings to the system (Fig. 15 G & F). The vulva or ostium bursac constitute the bursa copulatrix in female lepidoptera (Klots, 1956). The corpus bursae without containing a spermatophore is flattened dorso-ventrally and its walls are deeply folded (Fig. 15 H). It expands considerably following the insertion of a spermatophore, the shape of which has previously been described by Outram & Campion (1967). The tip of the spermatophore collum possesses elaborate horns in some species (Norris, 1932; Callahan, 1960) but in Diparopsis the collum tip is only slightly bent and possesses a small opening which comes to lie close to the seminal duct conveying sperm from the spermatophore to the spermatheca (Fig. 15 I & J). The other external opening of the female reproductive system is the oviporus or ostium oviductus (Fig. 15 F). The vagina extends outward from the oviporus, The paired accessory gland reservoirs
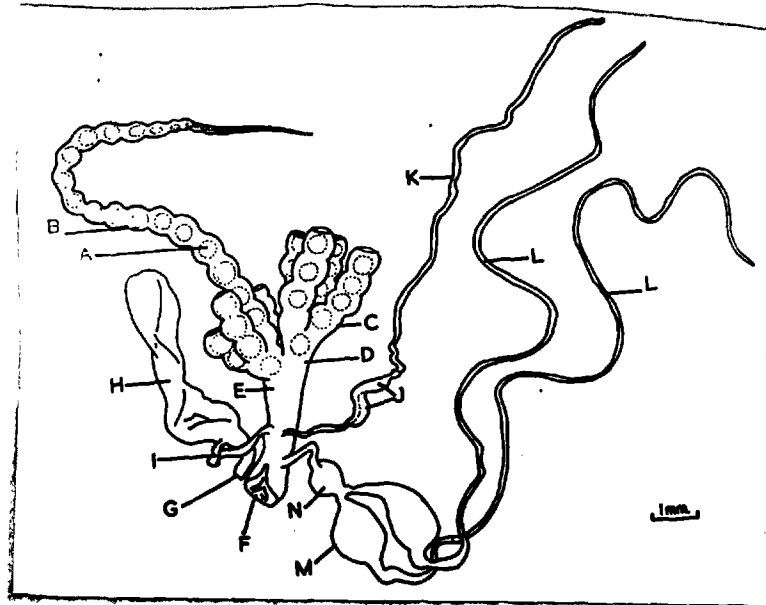
Fig. 15. Female reproductive system of Diparopsis.

A, mature egg; B, ovariole; C, calyx; D, lateral oviduct;
E, common oviduct; F, ostium oviductus; G, vulva;
H, bursa copulatrix; I, seminal duct; J, bilobed
spermatheca; K, spermathecal gland; L, accessory gland;
M, paired accessory gland reservoirs; N, duct of
accessory gland and common reservoir.

are orange-yellow in colour in living specimens, they are thin-walled and lie dorsally on either side of the vagina (Fig. 15 M). A tube, the accessory gland (Fig. 15 L) extends from each reservoir and is very long and narrow to become convoluted among other internal organs. The bilobed spermatheca (Fig. 15 J & D) fuses proximally to form the spermathecal duct, while the spermathecal gland is also long and convoluted (Fig. 15 K). The paired ovaries are pale green and held together in a compact mass by tracheae and fat-body. They occupy most of the abdominal cavity and since they exceed the length of the abdomen, they are reflected back on themselves, terminating in the third or fourth abdominal segment. Each ovary consists of four polytrophic ovarioles (Fig. 15 A & C). They adhere apically very close to each other. Proximal from the germarium is the vitellous region which contains a series of developing oocytes of gradually increasing size. Each developing egg is contained within an egg-chamber or follicle and consists of an oocyte and 5 nurse cells, or trophocytes (Fig. 16). The lateral oviducts (Fig. 15 D) fuse to form the common oviduct which is continuous with the vagina.

2. The effect of tepa-treatment on the gross structure of the reproductive systems of Diparopsis.

The effects of tepa-treatment applied to female Diparopsis after an interval of 3 days were quite pronounced. Fig. 16 shows

<u>Fig. 16.</u> Effect of injected doses of 10 µg of tepa applied to

female <u>Diparopsis</u> on (a) the developing oocytes,

(b) mature eggs when examined 3 days post-treatment;

compared with effects on an untreated female

(c & d) of similar age. Sections stained with Delafield's

haematoxylin. A, nurse cells; B, degenerating nurse cells;

C, egg; D, follicular cells; E, chorion.
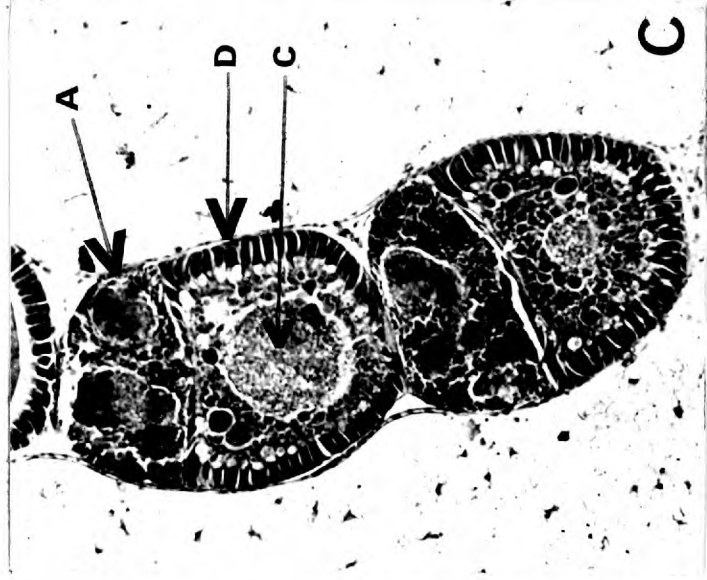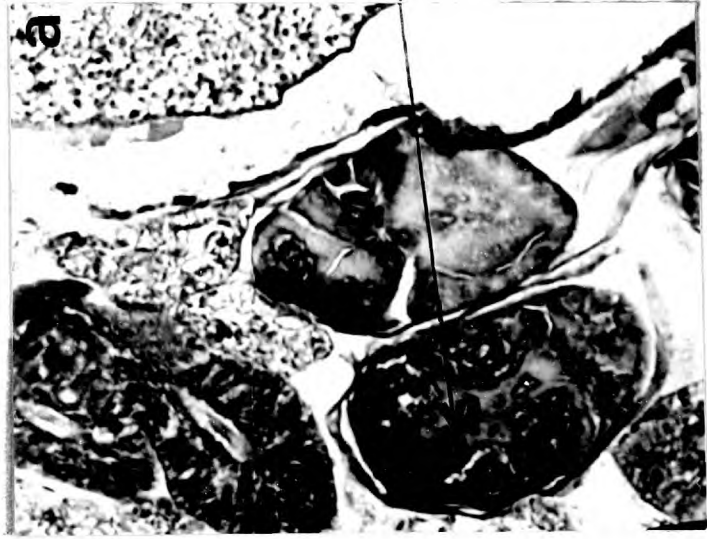
Fig. 17. Effects of injected doses of 10 μg of tepa on the reproductive

system when applied to male Diparopsis and examined 3 days

post-treatment; (a) Details from L.S. sections of the

abdomen of a treated male (b) L.S. of abdomen of an

untreated male of similar age. Stained with Delafield's

haematoxylin.

A, T.S. upper vas deferens; B, oblique sections through bulbous

region of seminal vesicles filled with sperm; C, oblique

sections through ductus duplex; D, T.S. ductus simplex;

E, T.S. chitinous simplex; F, T.S. accessory gland.
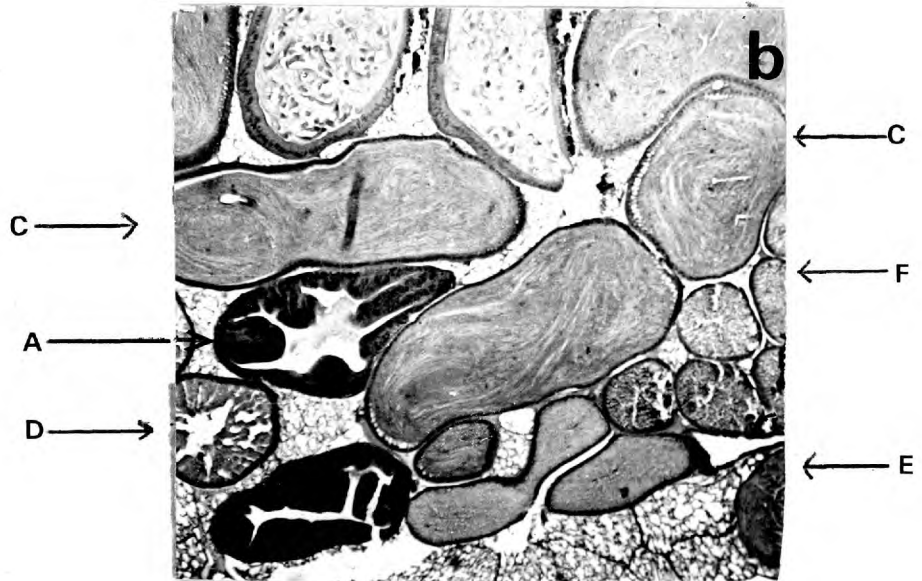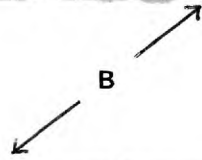
sections through both developing oocytes and mature chorionated
eggs of untreated insects compared with the same stages from
treated insects. The young oocytes from the treated insect
have already started to degenerate, the trophocytes being
particularly affected. More remarkable was the contraction
in size of some of the already mature eggs, as evidenced by
the large gap between the chorion and the egg proper. The
control eggs were of normal size.

Longitudinal sections of abdomens of male Diparopsis
failed to show any abnormalities as the result of tepa
treatment (Fig. 17).

Sections of testes from tepa treated and untreated
Diparopsis and Autographa are shown in Fig. 18, in neither
instance was damage noted as a result of treatment. Only
mature sperm were seen in the testes of Diparopsis; although
a greater preponderance of earlier stages of spermatogenesis
occurred in Autographa.

3. The embryological development of Autographa and Diparopsis
   eggs fertilised by sperm from tepa sterilised males.

A histological examination was made of 33 eggs. After 3
days incubation, most of the eggs from untreated parents showed
well differentiated alimentary-canal, well-defined nerve-chord
and a deliminated hypodermis, while the frontal sacs from which
the optic discs are derived had made their appearance (Fig. 19A&B).

Fig. 18. Effects of injected doses of 10 µg of tepa applied to male Diparopsis and Autographa on the testes when examined 3 days post-treatment. (a) and (b) treated and control sections of Diparopsis testes; (c) and (d) treated and control sections of Autographa testes. Stained with Delafield's haematoxylin.

In Autographa all stages of spermatogenesis can be seen, whereas in Diparopsis mostly mature sperm are found and the septa between the follicles have degenerated.
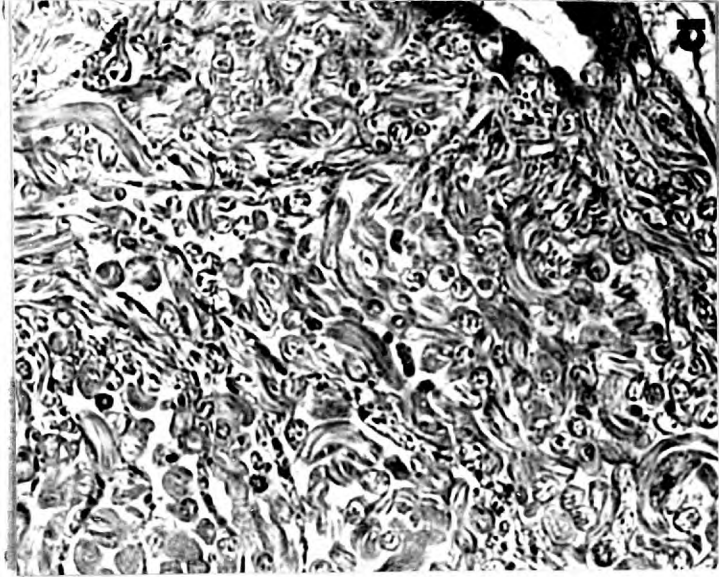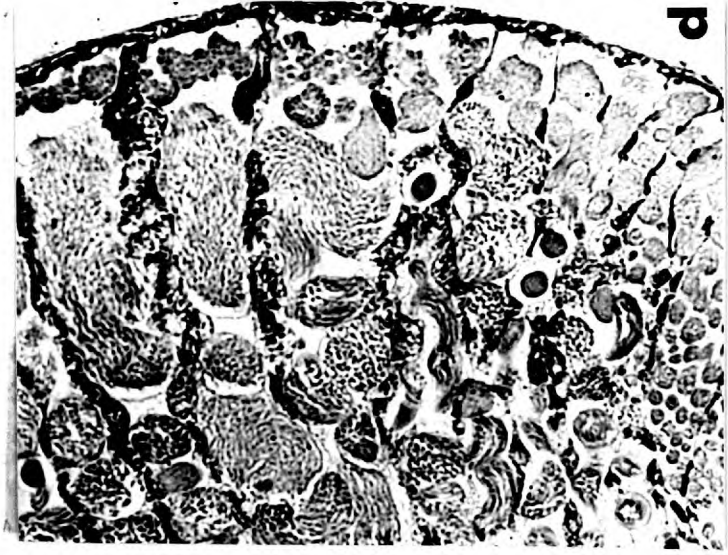
In contrast most of the eggs fertilised with sperm from tepa treated males showed signs of arrested development at a very early stage. Autolysis had occurred with the disintegration of the yolk into tiny droplets (Fig. 19 C & D). A few eggs showed signs of further development, although one was irregular in shape and another with organs less well differentiated than the controls (Fig. 19 E & F).

The level of embryological development in the eggs of Diparopsis following treatment of male moths with varying doses of tepa, metepa and apholate is shown in Table 41. With all three chemosterilants considerable embryological development without hatch occurred in the lower dosage levels.

4. Histochemical tests.

Tests for alkaline phosphatase activity were negative for incubation periods of 4 and 8 hours. After a 24 hour incubation period pronounced phosphatase activity was noted. An example of the enzyme distribution is shown in Fig. 20, and was found particularly associated with the mature sperm. Heavily stained sperm in the lumen of the vas deferens for example can be clearly seen. The differences in staining intensity of testicular preparations from tepa treated and untreated moths incubated together under identical conditions is shown in Table 42. The results from five replicate tests suggest a consistant reduction of phosphatase activity as a

Fig. 19. Effects of injected doses of 10 μg of tepa applied to

male _Autographa_ on the embryological development of eggs

incubated for 3 days, after mating with untreated females,

compared with development in eggs from untreated parents.

(a) T.S. control egg   (b) L.S. control egg

(c) T.S. treated egg   (d) L.S. treated egg
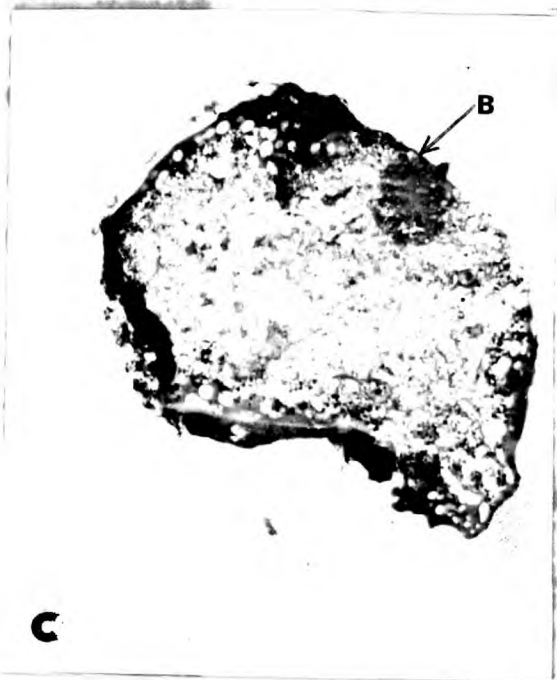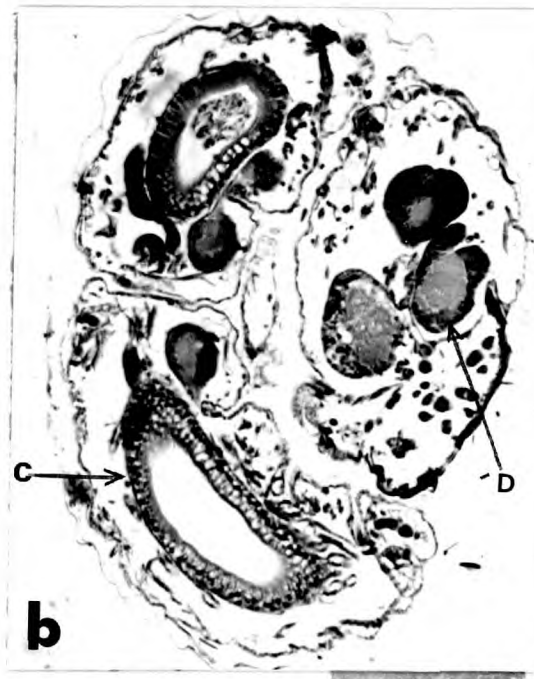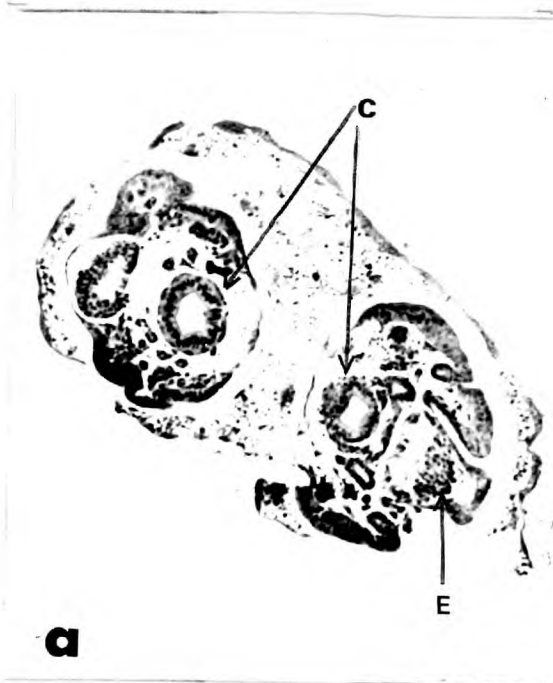
Fig. 19 (continued). (e) and (f) treated eggs.

A, yolk droplets; B, disintegrating nuclear material;

C, alimentary canal; D, developing brain;

E, nerve chord. Stained with Delafield's haematoxylin

and eosin.

Fig. 20. Section of Diparopsis testes to show the distribution of alkaline phosphatase activity. The beginning of the upper vas deferens can be seen on the right, in the lumen of which are heavily stained sperm. The cell walls of the vas deferens are virtually unstained.

A, sperm; B, lumen of vas deferens;

C, cell wall of the vas deferens.

Table 41. Relationship between doses of apholate, metepa and tepa applied by injection to _Diparopsis_ males and development in non-hatching eggs.

Chemosterilant	Dose (µg)	Eggs laid	Eggs hatching (white)	Non-developing eggs (green)	Developing eggs (grey)	% Non-developing eggs	%developing eggs	% total sterility
apholate	2.5	261	16	84	160	31.2	61.3	97.4
	5.0	138	0	86	52	62.3	37.7	100
	10.0	166	0	162	4	97.6	2.4	100
	20.0	208	0	205	3	98.6	1.4	100
metepa	2.5	295	77	15	203	5.1	68.8	73.9
	5.0	114	19	33	62	23.6	44.3	83.3
	10.0	120	4	13	103	10.8	85.8	96.7
	20.0	80	0	55	25	68.6	31.4	100
	40.0	130	0	130	0	100	0	100
tepa	0.5	136	90	19	27	14.0	19.9	34
	1.0	426	80	133	213	31.2	50.0	81.2
	2.0	391	0	358	33	91.6	8.4	100
	4.0	461	0	456	5	98.1	1.1	100
	5.0	286	0	286	0	100	0	100

Table 42. The staining intensities measured by a scanning microdensitometer[+] of sections

of testicular preparations of _Diparopsis_ for alkaline phosphatase activity.

Testes dissected from moths treated 3 days before fixation with 10 µg of tepa

compared with testes from untreated insects.

Replicate	Mean value ex-[*] treated sections	Mean value ex-[*] control sections	Difference (T-C)	% difference (T-C)
1	2260.0	2257.6	+2.4	
2	533.4	418.7	-114.7	
3	1401.1	3036.6	-1635.5	
4	1605.0	2096.0	-491.0	
5	67.0 [x]	119.0	-52.0	
Totals		7927.9	-2290.8	-16.6

[*] Mean of 20 readings, 10 from periphery and 10 from centre of section.
[+] x40 objective, 100% transmission.
x old block, retained in refrigerator for 2 weeks before sectioning.

result of tepa treatment with a mean reduction of 16.6%.

The staining intensities of sperm heads treated by the Feulgen method are shown in Table 43. The results were subject to considerable variation while the mean values from 5 replicate tests gave only a slight increase of 7.6% in staining intensity from the material from tepa treated males compared with the controls. The results from a single PAS test similarly showed a slight increase in staining intensity of 5.8% as the result of tepa treatment.

5. The agglutination effect of tepa on mature sperm.

The results of the observations on the agglutination effect of tepa, following the dissection and histological examination of 32 spermathecae of mated Diparopsis females are summarised in Table 44, and illustrated in Fig. 21. It is evident that severe agglutination occurred at an injected dose of 40 µg of tepa while at the 10 µg level, some agglutination occurred in 5 out of the 11 spermathecae examined.

6. Structure of the spermatozoa of Diparopsis.

Untill recently the structure and functioning of insect spermatozoa has been generally neglected. Recent progress has been reviewed by André (1962) and Thompson & Blum (1967) Smith.D. (1968) and Phillips (1970) when it was shown that a great diversity of structure occurred.

Table 43. The staining intensities measured by a scanning microdensitometer[+] of sections of sperm heads treated for Feulgen and PAS activity. Testes dissected from moths treated 3 days before fixation with 10 µg of tepa compared with testes from untreated insects.

Treatment	Replicate	Mean value * ex treated sections	Mean value * ex control sections	Difference (T-C)	% difference
Feulgen	1	8.81	1.31	+7.50	
	2	3.98	0.77	+3.21	
	3	1.39	2.15	-0.76	
	4	11.45	15.72	-4.27	
	5	11.76	12.13	-0.37	
	Total	37.39	32.08		
	Mean	7.47	6.41		7.64
PAS	1	6.06	5.40	+0.66	5.75

* Mean of 20 readings   5 from any one section.
+ x100 objective 70% transmission.

Table 44. To show the effect of tepa when injected into male _Diparopsis_ in causing

agglutination of sperm transmitted to female moths when stored in the spermatheca.

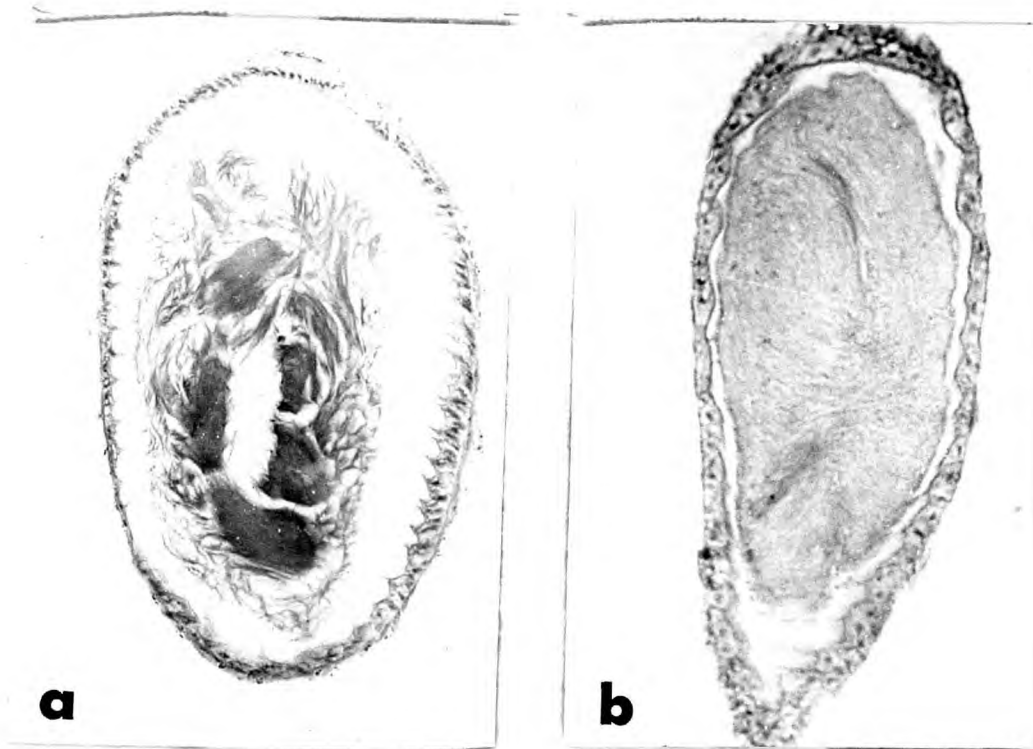| Treatment (μg)	No. of spermathecae examined histologically	No. of spermathecae containing severely agglutinated sperm	No. of spermathecae containing slightly agglutinated sperm	Total no. of spermathecae showing signs of agglutination in sperm	% agglutination
40	3	3	0	3	100
10	11	5	0	5	45
5	4	0	1	1	25
C	14	0	3	3	21

Fig. 21. The agglutination effect of sperm from tepa treated
male Diparopsis when stored in the spermathecae of
subsequently mated females.

(a) sperm from males injected with 40 µg of tepa

(b) sperm from untreated males.

Sections stained with Delafield's haematoxylin
and eosin.

Diparopsis 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 Ōura (1965) in _Bombyx mori_. From its size and position in _Diparopsis_ this may possibly be associated with the acrosome (Fig. 22 a).

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 (1959, 1962), who first described them in sperm of _Pieris_ and _Macroglossum_, arise from the cell membrane and are finely striated transversely. Phillips (1970) 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 recognisably 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 mitochondrial structures. (Fig. 22 c). They are derived during spermatogenesis from the aggregation of mitochondria into nebenkern, the material of which reorganises itself and elongates along the developing flagellum. (See the review by Phillips, 1970).
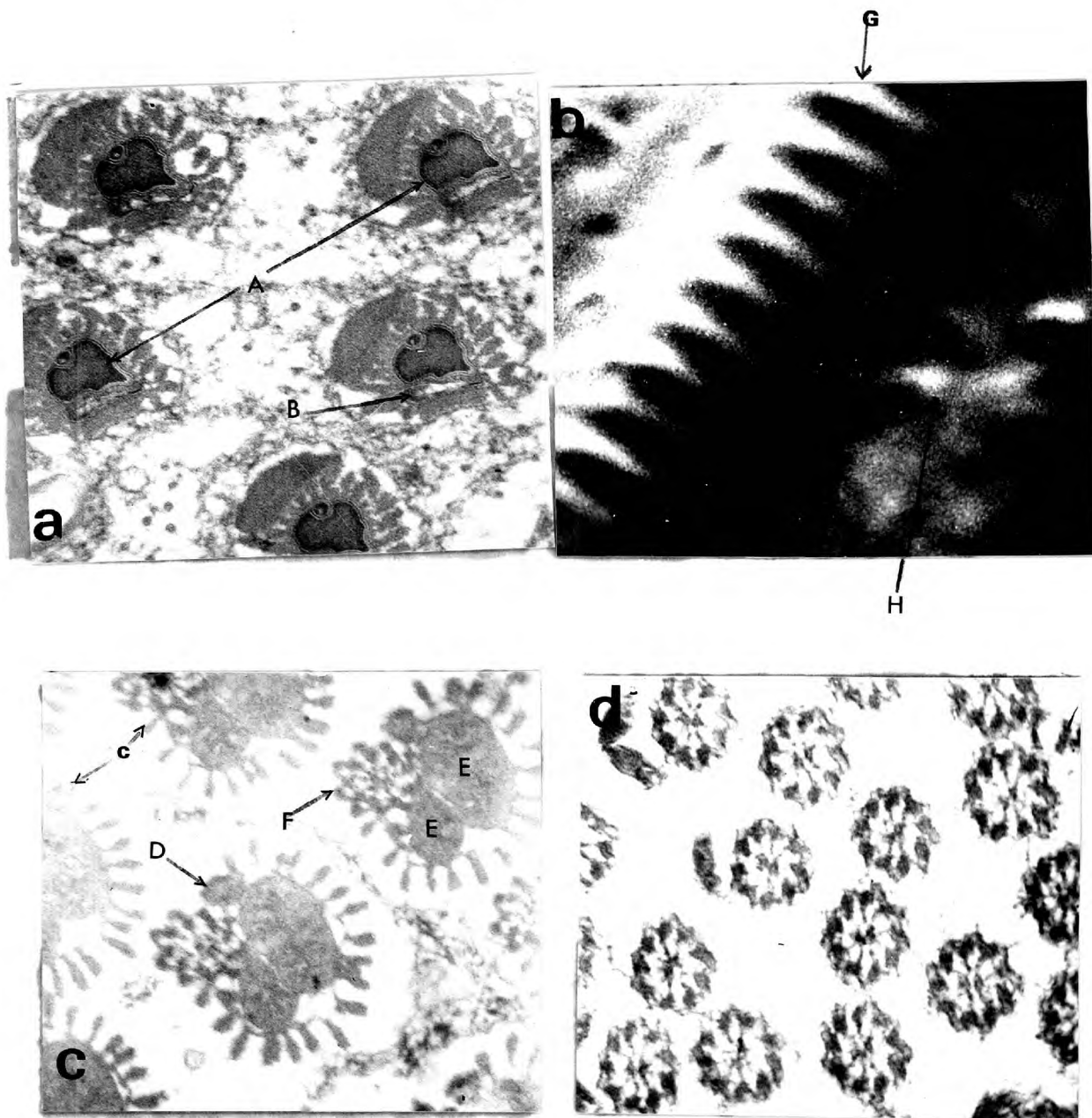
Fig. 22. To show ultrastructure of <u>Diparopsis</u> spermatozoa.

(a) T.S. through head region, (b) external view of

head region, (c) T.S. anterior tail region,

(d) T.S. posterior tail region. A, nuclear material;

B, tubular structure; C, appendices lacuniae;

D, reticular appendage; E, mitochondria; F, flagellum;

G, transverse corrugations.; H, partially collapsed
appendices.

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. 22 d). The flagellum possesses the familar organisation of two central fibrils with nine outer doublets. An additional nine single fibrils are closely associated with the outer ring of doublets.

When whole air-dried spermatozoa are observed in the scanning electron microscope, the delicate radial appendages cannot properly be seen since they have collapsed in the process of drying. If the preparation is shadow cast, regular transverse corrugations are revealed which may represent a close spiral organisation of the mitochondrial cortex (Fig. 22 b). Such a spiral organisation cannot be deduced from the thin sections by transmission electron microscopy

No evidence of damage to the delicate ultrastructure of the spermatozoa was observed three days after the injection of males with 10 μg of tepa, a treatment considerably in excess of the minimum sterilising dose.

DISCUSSION

It is clear that although the alkylating agent tepa had
a marked effect on developing oocytes and even mature eggs
in Diparopsis no gross damage to the testes or related organs
was noted. A similar conclusion was independantly reached
by Bulyginskaya (1967) from a study of the effects of thiotepa
and tretamine against several noctuiid moths. The shrinkage of
the mature eggs may indicate a secondary effect of the
chemosterilant on the endocrine system (see Part V). The
lack of effect on the male system following tepa treatment
is consistant with the fact that spermatophore production and
size was completely unaffected (Outram & Campion, 1967).
On the other hand Hamilton & Sutter (1969) claimed that
apholate orally applied to the Southern corn root worm
Diabrotica undecimpunctata howardi, caused a reduction in
accessory gland secretion.

However when male Diparopsis were injected with 10 µg
amounts of tepa a consistant reduction in phosphatase activity
associated with sperm occurred. A disturbance of enzyme
balance in the sperm almost certainly related to the mitochondria
could lead to a loss of motility. Reduction in motility was
further confirmed by the agglutination tests. Marked agglutin-
ation of sperm, a phenomenon associated with loss of motility,
occurred in all instances in transmitted sperm when males were
treated with 40 µg of tepa, while at the 10 µg level 45% of
the spermathecae examined contained agglutinated sperm.

Although such levels are in excess of the SD_{95} level for
tepa of 1.33 µg (Part I), these observations are only
approximations and therefore less easily detectable differences
in sperm immotility may be an important component of sterility
even at lower dosage levels.

Infertility in man and higher animals is generally
associated with a lowering of sperm DNA content, while a
lowering of sperm DNA content has also been associated with
a loss of sperm motility (Mann, 1964). From the limited tests
with Diparopsis it was shown that a slight increase in DNA
content measured by the Feulgen reaction had occurred following
tepa treatment. It is known that the molar absorptivity of
DNA increases on depolymerization or denaturation. Therefore
although the results for Diparopsis should be treated with
considerable caution, it is tempting to suggest that such a
denaturing effect could be the direct result of tepa treatment.

It was shown that in newly emerged male Diparopsis only
mature sperm were present in the testes. Tepa was a very
effective sterilising agent for such an insect (Part I).
Jackson & Schnieden (1968) reported that tepa and related
aziridines were most active against spermatozoa and spermatids
in rodents, while the sulphonic acid ester busulphan on the
other hand selectively suppressed early stages of spermatogonial
development in rodents. It is therefore not supprising that
busulphan was inactive against Diparopsis males (Part I).

It is also evident that embryo death can occur at any time

and is not necessarily confined to the early cleavage stages.

Where no egg development occurs at all, this may be attributed

to dominant lethality, although sperm inactivation may be

occurring which is preventing fertilisation. No damage to

the sperm ultrastructure was observed at a dose in excess of

the minimal sterilising dose and associated with a reduction

in phosphatase activity and the phenomenon of agglutination.

It would therefore appear unlikely that gross mechanical

damage to the ultrastructure would provide an important

component of sterility.

The presence of sperm in the spermatheca is the necessary

stimulus for oviposition; with the result that pheromone

secretion ceases and therefore the males made no further

mating attempts (Cottrell, 1967). A spermicidal agent causing

agglutination without attendant mutagenic hazard might therefore

be the kind of sterilising agent most suitable for such an

insect. Spermatogenesis has been virtually completed on adult

emergence and no recovery of fertility would therefore be

possible.

Earlier workers such as Yamane (1921) found that various

salts of aluminium, iron and lead caused agglutination in

frog sperm. The weak sterilising activity of some of the

organometal compounds tested against _Diparopsis_ males (Part I)

may therefore be due to such an effect. Indeed, Ascher _et al_

(1968) attributed the chemosterilising action of triphenyl tin
acetate against houseflies as a gradual kill and immobilisation
of sperm in the spermathecae of untreated females after mating
with treated males had occurred. Unlike the aziridines the
dose mortality and sterility regression lines for triphenyl tin
acetate against male Diparopsis were not parallel, (Part I),
which suggested an independant action on sperm not directly
related to a general reduction in cell metabolism.

PART V.

Possible relationships between chemosterilant action and

endocrine misfunction in insects.

INTRODUCTION

In Part I, of the candidate chemosterilants evaluated, apart from the aziridines, only the s-triazine hemel showed appreciable sterilising activity against male Diparopsis. The s-triazines tested against female Diparopsis failed to cause sterility but often increased the oviposition rate, a property also noted for the carbamate insecticide carbaryl when applied at sub-lethal doses. Reference has already been made to the species specificity of several non-mutagenic chemosterilants. To gain more insight into possible causes of such specificity, a series of s-triazines containing progressively less methyl substitution were evaluated for chemosterilant activity against male Diparopsis, and the results obtained compared with those reported for male houseflies by Chang et al (1968) and LaBrecque et al (1968).

The results for Diparopsis were also analysed for mating disfunction as a major component of the sterility effect. This meant that although a spermatophore was produced by the male the sperm apparently failed to reach the spermatheca of the female. Other examples of mating aberrations were studied in both sexes of moth as the result of treatment, including the increased oviposition rate in females, automatic spermatophore ejaculation in males and permanent copulation.

Reserpine, a well known tranquillising agent, caused complete inhibition of oviposition in houseflies which was associated with the induction of endogenous serotonin a

powerful pharmacologically active substance (Hays et al, 1969).
It was noted earlier that reserpine also caused a reduction
in oviposition together with some sterility in female Diparopsis
(Part I). A wealth of information on the pharmacology of
reserpine has been accumulated but its extremely complex
pharmacological picture has not yet been fully elucidated.
Some of its effects can be explained by depletion others by
liberation of endogenous substances (see review by Schlittler
1967).

Hinks (1967) suggested that one such substance, serotonin
might be implicated in initiating flight activity in certain
Lepidoptera through the mediation of the neurosecretory cells.

By means of spectrophotofluorometric methods, the release
of serotonin and other endogenous biogenic amines, as the result
of treating Diparopsis with reserpine, carbaryl and hemel was
investigated. From the results obtained an attempt was made
to provide a theoretical basis for the species specificity of
chemosterilant action so often exhibited by many non-alkylating
chemosterilants.

MATERIALS AND METHODS

1. Treatments

The standard method of application and conditions post-treatment described in Part I were employed. Where mating aberrations such as permanent copulation were observed, the insects were fixed in Bouin's fluid and dissected at a later convenient time.

2. Spermatophore formation as the result of treatment with s-triazines or insecticides.

Male _Diparopsis_ moths were treated by certain s-triazine chemosterilants and insecticides in the standard manner. They were subsequently held for a minimum of 3 hours in plastic sandwich boxes (11 x 6 x $3\frac{1}{2}$ in) in groups of 20. The number of extruded spermatophores was then counted. To facilitate closer observation of this phenomenon under a binocular microscope, immediately after treatment the moths were fastened to glass slides, ventral side uppermost, by means of a suitable adhesive (Stikem Special).

3. Spectrophotofluorometric analysis.

(A) _Serotonin_. The assay of serotonin in insect tissue followed the spectrophotofluorometric method described by Bogdanski et al (1956). A spectrophotofluorometer is capable

of activating compounds and measuring their emitted fluorescence
from 250 to 650 mµ. It uses a xenon lamp as a continuous
light source with one quartz monochromator to select the
activating light and another to select the emitted fluorescence.
A photomultiplier is used to produce data for identification
and quantitative assay. Such an instrument offers a
sensitivity of at least two orders of magnitude greater than
spectrophotometry and the additional specificity inherent
in two spectral requirements instead of one.

For estimation of serotonin in insect tissue 15 to 50
insects after various treatments were homogenised in a high
speed homogeniser for 15 minutes in 15 ml of 0.1 N hydrochloric
acid. The pH was then adjusted to approximately 10 with
anhydrous sodium carbonate and 5 ml of borate buffer pH 10
was added followed by 5 gm of sodium chloride and 25 ml of
n-butanol (purified by successive washings with 1 N NaOH, 1 N HCl
and two washings with water). The mixture was shaken for
15 minutes on a mechanical shaker and then centrifuged. The
butanol phase was transferred to another container together
with 50 ml of heptane (also purified by successive washings
with 1 N NaOH, 1 N HCl and two washings with water) and 3 ml
of 0.1 N hydrochloric acid and shaken for 10 minutes. The
mixture was centrifuged for 5 minutes and 2 ml of the acid
were added to 0.6 ml of concentrated hydrochloric acid in a
quartz cuvette. The solution was activated in an Aminco-Bowman

spectrophotofluorometer at 300 mμ and the fluorescence curve plotted from 270 to 600 mμ. The main fluorescence peak for pure serotonin at pH 1 was at 540 mμ. To determine the efficiency of the extraction procedure, known amounts of serotonin (as serotonin creatinine sulphate) were added to moth homogenates and the values obtained at 540 mμ compared with those from the pure material when also passed through the extraction train. According to Bogdanski et al (1956), 0.1 μg of serotonin can be detected by this method. Apart from Diparopsis, approximate levels of serotonin were also determined for the adult cotton stainer Dysdercus fasciatus, the yellow mealworm Tenebrio molitor and the housefly Musca domestica.

A fluorescent peak at 340 mμ (uncorrected) was also consistantly found for Diparopsis which increased markedly after certain treatments. According to Duggen et al (1957) this would be due to the presence of catecholamines, including adrenaline, noradrenaline and dopamine. This characteristic was used as such by Bertler et al (1958) to determine the total catecholamines of adrenals in rabbits.

(B) Catecholamines. These substances fluoresce because of their phenolic nature and is therefore not specific. A preliminary characterisation of catecholamines in Diparopsis was determined by use of the trihydroxyindole reaction

described by Udenfriend (1962), following the experimental
procedure of Shore & Olin (1958).

Groups of insects weighing from one to five grams were
homogenised for 15 minutes in 15 ml 0.01 N HCl. The homogenate
was transferred to a 150 ml centrifuge bottle containing 8 gm
of solid NaCl and 60 ml of butanol. After shaking for
1 hour on a shaking apparatus, the bottle was centrifuged and
a 40 ml aliquot of the butanol layer transferred to another
bottle containing 5 ml of 0.01 N HCl and 70 ml of heptane.
The bottle was shaken for 5 minutes and then centrifuged.
The aqueous phase was analysed fluorometrically for catecholamines.

The catecholamines are converted to highly fluorescent
trihydroxyindoles. A 1-3 ml sample of the extract was transferred
to a test-tube and either one ml of pH 5 or pH 3 acetate, buffer
was added followed by 0.1 ml of iodine reagent (1.27 gm of iodine
dissolved in 100 ml of absolute ethanol). After six minutes the
excess iodine was destroyed by the addition of 0.2 ml of
thiosulphate solution (1.24 gm $Na_2S_2O_3 5H_2O$ dissolved in 100 ml
of water). One ml of alkaline ascorbate solution was then added
(made immediately before use by adding 1 volume of an aqueous
solution of ascorbic acid (10 mgm/ml) to 2 volumes of 5 N NaOH)
and 1.5 ml of water. After 45 minutes the solution was
activated at 400 mμ and the resulting fluorescence spectrum
determined. Correction for non-catechol fluorescence was
measured in a sample extract by adding all the reagents but

reversing the order of the iodine and thiosulphate.

The fluorescence of the product derived from noradrenaline is decreased when oxidation is carried out at a low pH, while the fluorescence derived from adrenaline is only slightly affected. Such a difference in oxidation at pH 3 and pH 5 can serve a convenient means for the differential assay of the two catecholamines.

For the determination of free catecholamines in terms of noradrenaline equivalents, the following formula was used:-

μg/15 ml of homogenate = $\dfrac{\text{reading of 1.5 ml extract at pH5}}{\text{reading of 1.5 ml NA internal standard}}$
$(\mu$g/ml$)$

$$5 \times \dfrac{\text{Volume of butanol added to homogenate}}{\text{Volume of butanol analysed}}$$

RESULTS

1. The effects of varied methyl substitution on the chemosterilant activity of a series of s-triazines applied to male Diparopsis.

The effects of varied methyl substitution in a series of s-triazines applied to male Diparopsis at injected doses of 40 μg compared with the reported chemosterilant activity against houseflies are shown in Table 45. Appreciable sterility expressed as "% sterile eggs" was obtained for several members of the series, although the compound most active against male houseflies N^2,N^2,N^4,N^4 tetramethyl melamine was completely inactive against male Diparopsis. On the other hand the parent tri-amino compound melamine which was virtually inactive against male houseflies, showed marked sterilising activity against male Diparopsis. An appreciable level of mating aberration was also seen throughout the series, expressed as permanent copulation or automatic spermatophore extrusion (see below). This latter phenomenon was particularly noted several hours after treatment with N^2,N^4,N^6-trimethyl melamine and N^2,N^2,N^4,N^6-tetramethyl melamine, both of which were shown to be metabolites of hemel in the housefly by Chang et al (1968).

Table 45. The effect of varied methyl substituents in a series of s-triazine hydrochlorides on sterility and mating disfunction by treating male _Diparopsis_ with injected doses of 40 µg in aqueous solution (20-25 replicates per compound).

Substituent groups *				% 4-day** mortality	% mating	% sterile mating	% spermatophore formation in unmated males	% ** sterile eggs	Sterilising+ activity in houseflies
R	R₁	Rᵢᵢ							
N(CH₃)₂	N(CH₃)₂	N(CH₃)₂	HCl	9	80	37.5	0	7.3	11.0 (µg/fly)
N(CH₃)₂	N(CH₃)₂	NHCH₃	"	10	28	33.3	0	56.0	10.3
N(CH₃)₂	NHCH₃	NHCH₃	"	25	24	33.3	11.5	61.0	14.1
N(CH₃)₂	N(CH₃)₂	NH₂	"	7	48	0	0	4	5.5
NHCH₃	NHCH₃	NHCH₃	"	28	100	11.1	24	0	5.3
N(CH₃)₂	NHCH₃	NH₂	"	10	115	20	2	17	28.6
NHCH₃	NHCH₃	NH₂	"	11	56	14	2	23	v/sl
N(CH₃)₂	NH₂	NH₂	"	0	67	12.5	0	17.6	sl
NHCH₃	NH₂	NH₂	"	6	90	22.2	0	32	nil
NH₂	NH₂	NH₂	"	5	90	58	3	42.5	sl

* The basic s-triazine structure is shown in Part I - Fig. 6.

** Adjusted for control mortality and sterility by Abbot's formula.

+ The first 6 compounds in the series were injected into male houseflies and the figure shown is the LD95 (ug/fly) (Chang et al 1968). The remaining 4 compounds were assayed by mixing them with the adult diet (Labrecque et al 1968).

2. <u>Mating aberrations in male Diparopsis after</u>
   <u>treatment with either certain s-triazines or carbaryl.</u>

In some instance following male treatment, although apparently normal mating had occurred, low numbers of eggs were laid characteristic of unmated females and these were all sterile. Other mated females laid larger numbers of eggs and these were invariably all fertile. In many Lepidoptera the presence of sperm in the spermatheca is the major stimulus for oviposition and this was also found to be true in the case of <u>Diparopsis</u> by Cottrell (1967). It was therefore suspected that where a so-called sterile mating had occurred, although a spermatophore had been transferred, the sperm had failed to reach the spermatheca.

The results presented in Table 46 shows the mean number of eggs oviposited from sterile, fertile and unmated females after treatment of male moths with hemel HCl and carbaryl. The similarity in the oviposition rate of sterile mated females and unmated females strongly supports the view that sperm had failed to reach the spermatheca. In Table 47 the incidence of such sterile matings are shown following topical application of male moths with sub-lethal doses of carbaryl and injected doses of hemel HCl.

In both instances a marked increase in mating aberrations was apparent compared with the controls. Mating disfunction

Table 46. The mean number of eggs produced from sterile matings, fertile matings and unmated females after treating males with injected doses of 40 µg of hemel HCl or topically applied doses of 0.2 µg of carbaryl (minimum of 20 replicates).

Treatment	Mean eggs ex-sterile matings	Mean eggs ex-fertile matings	Mean eggs ex-unmated females
Hemel HCl	19.6	129.7	10.8
Carbaryl	22.0	108.6	9.0
Controls	-	86.8	7.6

Table 47. Mating disfunction in Diparopsis following treatment of male moths with sub-lethal doses of carbaryl applied topically in acetone or aqueous injected doses of hemel HCl.

Treatment	No. of matings	No. of sterile* matings	% sterile matings
Carbaryl (0.05-0.2µg)	19	10	53
Hemel HCl (40µg)	22	6	29
Controls	29	1	3

* A sterile mating is defined as one where either no or a few sterile eggs are oviposited or where permanent copulation occurs.

was also sometimes expressed as permanent copulation. In such instances the spermatophore collum produced by the male was too long to be disengaged from the female and the partners were joined for life by the cement of the spermatophore cap; which normally fastens the spermatophore into position. An example of permanent copulation is illustrated in Fig. 23.

Figure 23 To show abnormal position of spermatophore collum when permanent mating occurs.

A	Spermatophore corpus	D	Spermatophore cap
B	Spermatophore collum	E	Male claspers
C	Bursa copulatrix	F	Cuticular ductus simplex
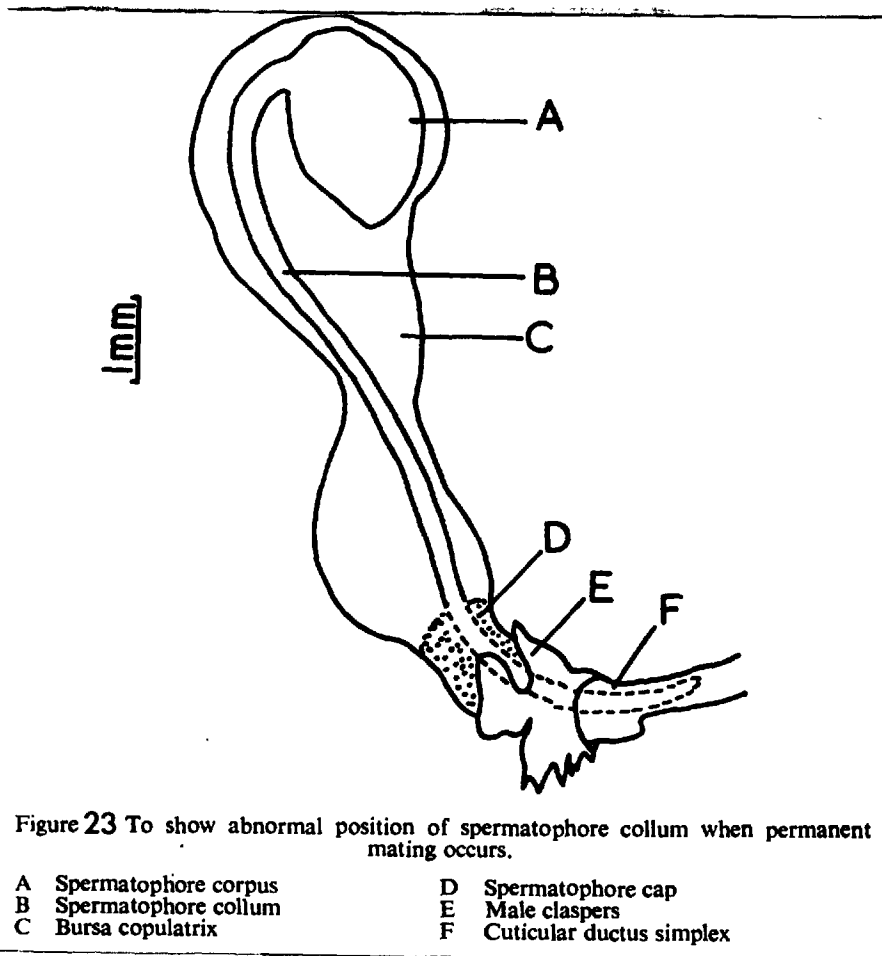
3. Spermatophore formation in Diparopsis as a result of treatment with certain s-triazines and insecticides.

Another phenomenon noted after application of some of the s-triazines listed in Table 45 was the automatic discharge of a crude spermatophore in the absence of a female moth. This effect was also seen after application of topical doses of carbaryl (Table 48). Unlike the s-triazines the effect with carbaryl only occurred at doses causing appreciable mortality within a few hours. A range of other insecticides, topically applied were also tested for this effect including γ-BHC, DDT, diazinon, dichlorvis, dieldrin, menazon, physostigmine and a mixture of crude pyrethrins. Only physostigmine, like carbaryl, a carbamic acid ester, gave comparable results (Table 48). The process of induced spermatophore formation is illustrated in Fig. 24. Five to ten minutes after treatment the claspers were fully extended and vigorous copulatory movements occurred (Fig. 24 a & b). A clear viscous secretion gradually appeared from the tip of the aedeagus (Fig. 24 c) after 30-40 minutes, which was accompanied by a slight eversion of the endophallus. Further secretions occurred to form a semi-spherical mass (Fig. 24 d) which gradually hardened on the outside (Fig. 24 e) and was sometimes moulded into a hollow sphere by the piston-like action of the aedeagus. After a total of 50-60 minutes, the endophallus had completely everted and was pulsating rhythmically, while the spermatophore collum was slowly extruded (Fig. 24 f).

Table 48. The effect of various insecticides when applied topically on the induction of

spontaneous spermatophore formation in male <u>Diparopsis</u>.

<u>Insecticide</u>	Total insects treated	Dose range (µg)	Total number spermatophores	% spermatophores
BHC	80	2.5-20	0	0
DDT	80	1-10	0	0
Dichlorvis	60	0.25-5	0	0
Menazon	100	1-5	0	0
Dieldrin	80	2.5-20	1	1.3
Diazinon	80	10-40	7	8.7
Pyrethrins	100	3-50	10	10
Physostigmine	60	5-20	22	26.6
Carbaryl	60	5-20	23	38.3

Fig. 24. Stages in the formation of a rudimentary spermatophore in
          Diparopsis following treatment with carbaryl at a topically
          applied dose of 5 μg.

          a, 10 minutes post-treatment; general copulatory movements
          occurring.

          b, 10-15 minutes post-treatment; aedeagus fully extended.

          c, 20-40 minutes post-treatment; a clear viscous secretion is
          beginning to appear from the tip of the aedeagus.

          d, 20-40 minutes post-treatment; the secretory sphere
          increases in size.
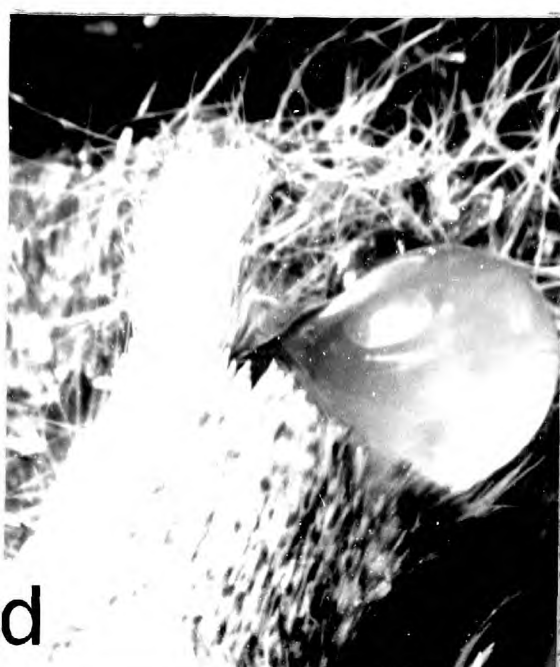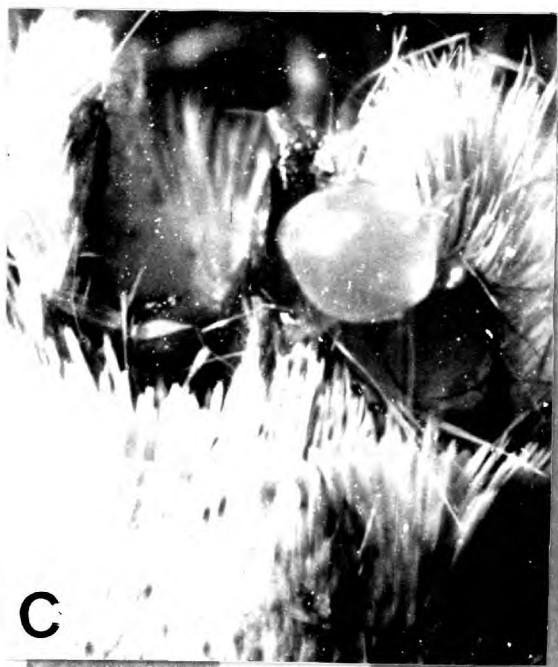
Fig. 24(continued).

e, 30-50 minutes post-treatment; secretions are beginning to harden.

f, 50-60 minutes post-treatment; the endophallus has completely

everted and is contracting rhythmically, accompanied by the

gradual extrusion of the spermatophore collum.

g, 90-100 minutes post-treatment; the full length of the collum is

extruded. Cap secretion is forming at the base of the spermatophore.

h, fully formed spermatophore with cap secretion removed.

After a total of 90-100 minutes, the full length of the collum had been drawn out. Further secretions occurred this time of a rubbery-life consistancy presumed normally to form the spermatophore cap (Fig. 24 g). Frequently this latter secretion and the seminal fluid became mixed. The sperm would normally pass into the corpus, but in the artificial process described, this was prevented both by the limited expansion of the corpus and by the twisting of the collum (Fig. 24 h).

4. Increased oviposition in female Diparopsis after treatment with certain s-triazines and carbaryl.

A further example of an aberration in the reproductive process as the result of treatment with certain s-triazines or carbaryl was an increased rate of oviposition in female Diparopsis. The results for sub-lethal doses of carbaryl and for two s-triazines at an injected dose of 40 µg are shown in Table 49. The increase in oviposition was tested statistically by the Whitney-Mann U test and was found to be significant for all three compounds.

Table 49. The effect of carbaryl applied topically and two s-triazine chemosterilants applied by injection on the fecundity of female Diparopsis.

Treatment *	Number of mated females	Mean eggs per mated female	% increase in oviposition rate	U value	P
Carbaryl (0.05-0.20µg)	31	122	107	277	.0007
Control	11	59			
Hemel HCl (tris-dimethylamino-s-triazine) (40µg)	6	135	58.8	14	.091
Control	8	85			
2,4,diamino-6-morpholino s-triazine (40 µg)	34	104	55.2	493	.0013
Control	15	67			

* No marked lethal effect or sterility effect was noted.

5. The effect of reserpine on reproductive processes in Diparopsis.

The effects of reserpine on both male and female Diparopsis are shown in Table 50. Injection of 1 µg or more completely inhibited mating in male moths. Against female moths doses of 1, 5 and 10 µg progressively increased the percentage sterility of the eggs laid while a reduction in oviposition was noted.

6. Spectrophotofluorometric analysis.

(A) Serotonin. Extraction of up to 5 gm of Diparopsis adults of either sex failed to demonstrate the presence of unbound serotonin (Table 51 and Fig. 25 A). The addition of known amounts of the biogenic amine to moth homogenates were however readily detected (Fig. 25 A), while a comparison with values at 540 mµ obtained with standard solutions also passed through the extraction train, showed that the extraction procedure was highly efficient (Fig. 26). A high level of unbound serotonin of 4.3 µg/grm was found in adult Dysdercus; a much lower level of 0.9 µg/gm was found for Musca. No serotonin was detected in adult male Tenebrio (Table 51). Trace amounts of serotonin were detected in female Diparopsis, following reserpine treatment applied by injection 24 hours earlier (Fig. 25 B).

Table 50. The effect of reserpine applied by injection to male and female _Diparopsis_.

Treatment (ug)	Sex treated	Number treated	Number mating	% mating	Eggs laid ex-mated females	Mean eggs per mated female	% hatch
20	female	25	0	0	-	-	-
10	female	22	10	45	502	50	24
5	female	17	8	46	737	92	58
1	female	26	6	23	424	71	60
5	male	20	0	0	-	-	-
1	male	27	0	0	-	-	-
Controls		16	7	44	714	102	95

Table 51. Approximate amounts of unbound scrotonin (µg/gm) found in adults of various insect species. (Values from mean of two replicates.)

Insect	Sex	Wt of insects extracted (gm)	Fluorescence value at 540 mµ	Total equivalent scrotonin (µg)	Scrotonin as µg/gm of insect
Diparopsis	males	5	0.9	not detected	<0.18
	females	5	1.2	not detected	<0.18
Dysdercus	both sexes	3	4.3	4.9	1.63
Musca	both sexes	1	1.84	0.9	0.9
Tenebrio	males	3	0.9	not detected	<0.3

Fig. 25 A. Fluorescence spectra of <u>Diparopsis</u> homogenates
extracted for serotonin. Excitation maximum 300mμ.
x-x = extraction of 5 gm male moths.
o-o = extraction of 5 gm female moths.
●-● = extraction of 1.5 gm male moths + 10 μg
serotonin standard.

B. Fluorescence spectra of female moths extracted
for serotonin 24h post treatment by injection
with 10 μg aliquots of reserpine, compared
with untreated moths.
1.5 gm of moth homogenate extracted in both
instances.
●-● = reserpine treatment
x-x = controls.

ARBITRARY UNITS

A

B

MU

350   400   450   500
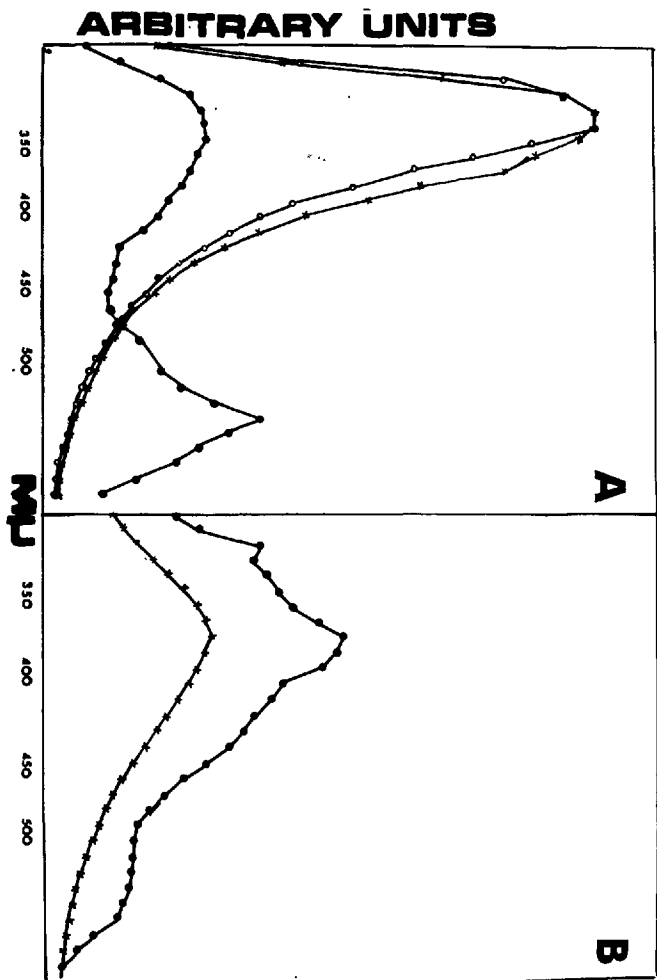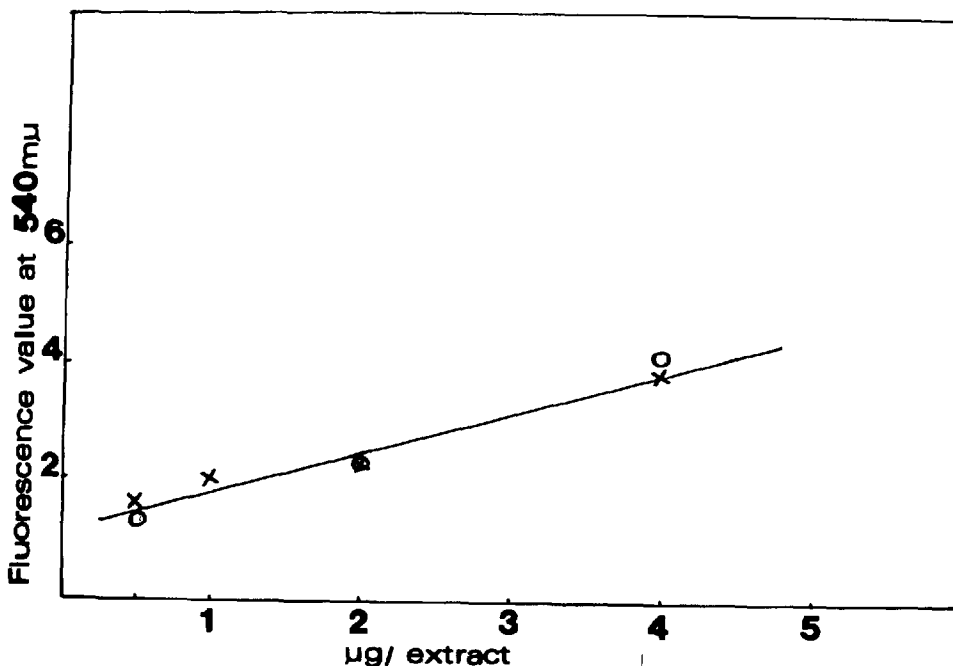
Fig. 26. To show (a) the linear relationship between levels
of serotonin added to <u>Diparopsis</u> homogenates and
the values obtained at 540 mμ; (b) the efficiency
of the extraction procedure.
x-x = values for serotonin added to moth
homogenates.
o-o = values for serotonin standards passed through
extraction train.

Treatment of either sex of <u>Diparopsis</u> with either 40 μg of

injected doses of hemel HCl or topically applied 5 μg doses of

carbaryl failed to induce the release of endogenous serotonin,

although a marked increase in a fluorescent peak at 340 mμ

was noted (Fig. 27 a & b).

On the instrument used in this study the catecholamines

adrenaline, noradrenaline and dopamine

fluoresce   at 340 mμ when activated at 290-300 mμ.

Fig. 27 a. The effect of injected doses of 40 µg hemol HCl on the
fluorescence spectra of (A) male Diparopsis extracts and
(B) female Diparopsis extracts, using the serotonin extraction
procedure. All moths were one day old at the time of
treatment; 15 moths per treatment. Excitation maximum 300 mµ.
x-x = 1 day post-treatment.   •-• = 3 days post treatment.
o-o = 6 days post-treatment.

b. The effect of topically applied 5 µg doses of carbaryl on
the fluorescence spectra of (A) male Diparopsis extracts
and (B) female Diparopsis extracts; using the serotonin
extraction procedure. All moths were one day old at the
time of treatment; 15 moths per treatment.
Excitation maximum 300 mµ.
•-• = 1½ hours post-treatment. o)o = 4 hours post-treatment.
x-x = controls.
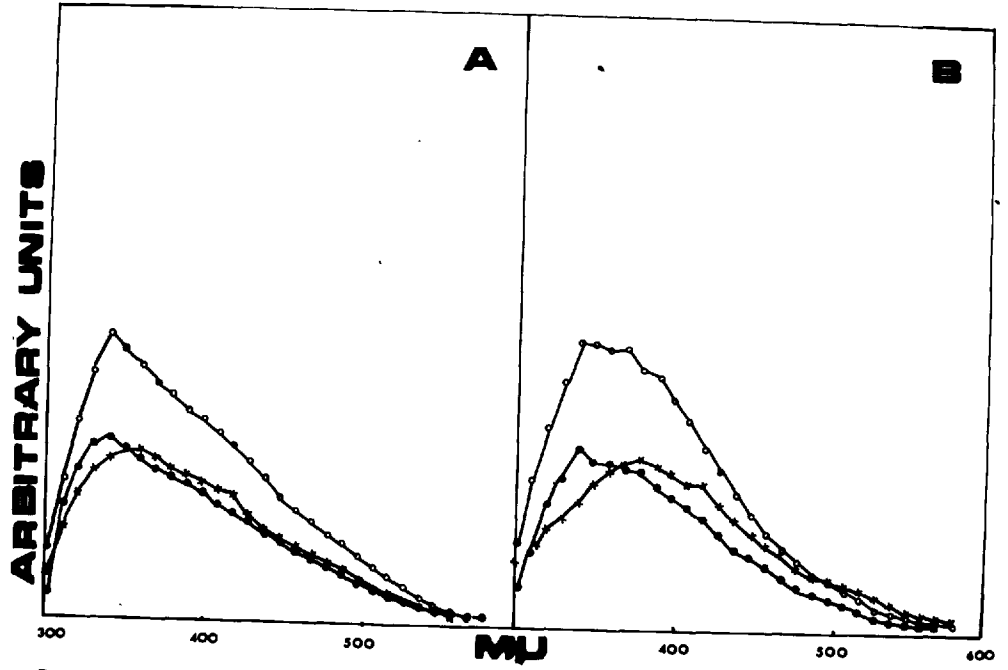
FIG 27a
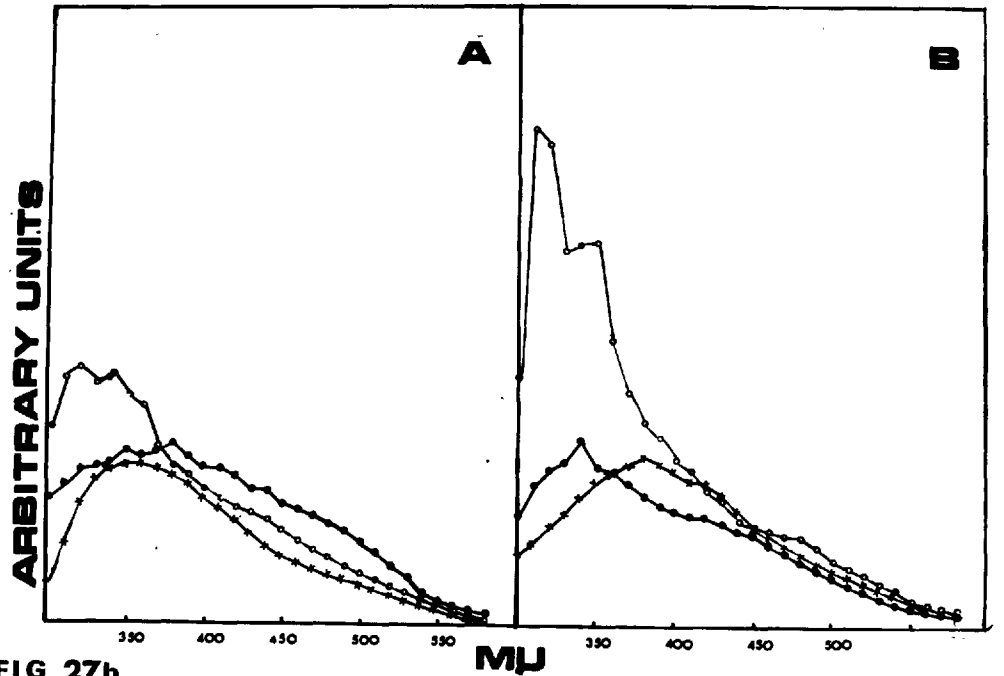
FIG 27b

Since such pharmacologically active substances could also be carried through the extraction process described for serotonin, attempts were made to confirm whether these substances were indeed present in the insect by a more specific method.

(B) Catecholamines. Fig. 28 shows the activation and fluorescence spectra of iodine oxidation products at pH 5 using male moth extracts alone and with added noradrenaline, compared with noradrenaline standard. The spectra in all instances are virtually identical.
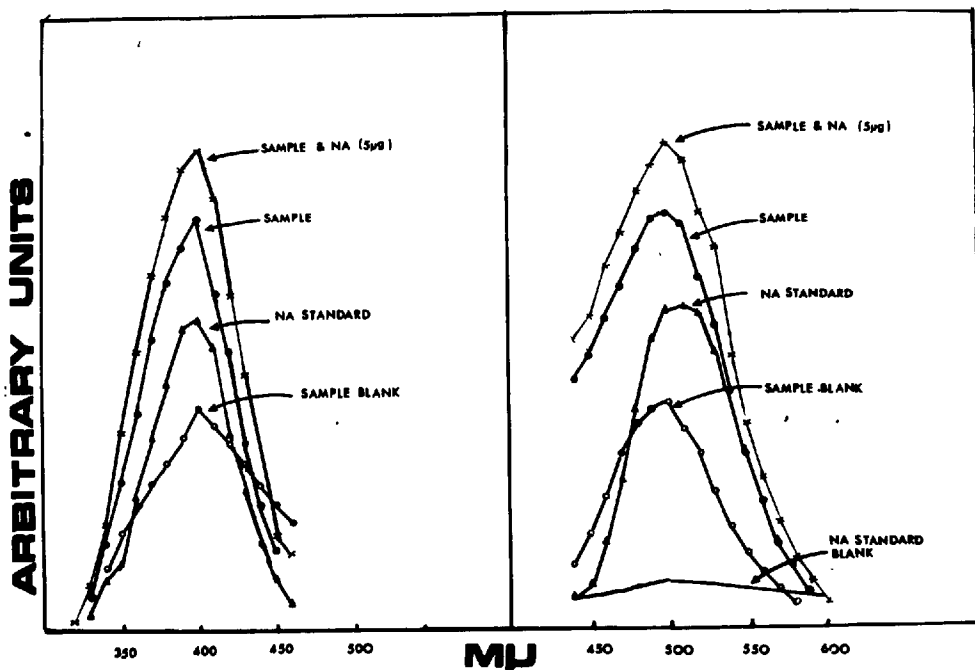
Fig. 28. Activation spectra (left) and fluorescence spectra (right) for catechols of male Diparopsis compared with noradrenaline alone and added to samples of moth homogenate, after trihydroxyindole reaction.

The fluorescence exhibited at pH 3 when oxidation of noradrenaline was negligible (Fig. 29), showed that virtually no adrenaline was present in the moth homogenates.
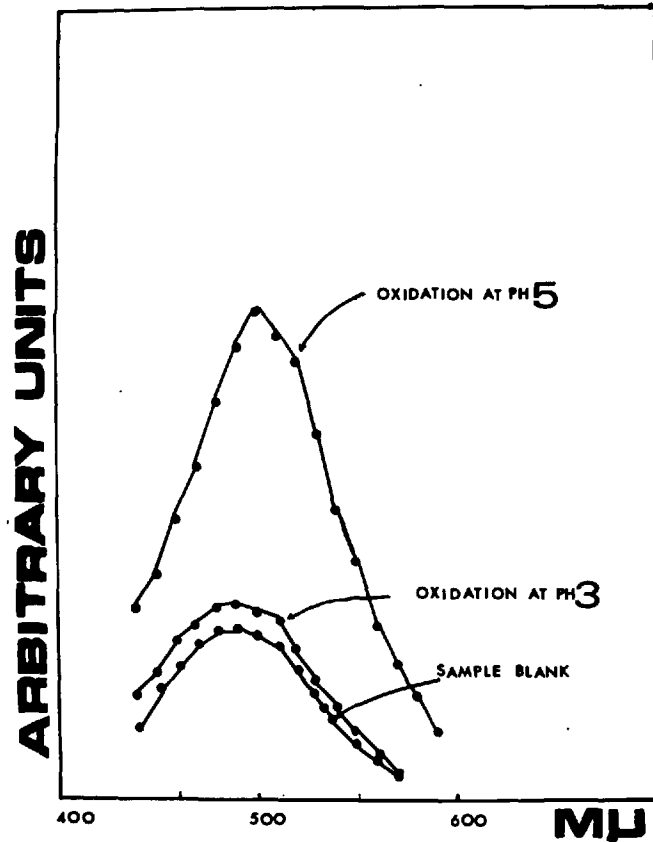
Fig. 29. The effect of pH on the fluorescence spectra of male Diparopsis homogenates after the trihydroxy-indole reaction. Excitation maximum 400 mμ.

The values for free catecholamines found in male Diparopsis in terms of noradrenaline (NA) equivalents are shown in Table 52.

Table 52. Concentration of noradrenaline (NA) in _Diparopsis_
male moths 2-4 days old and the recovery of some
material added to 15 ml aliquots of tissue
homogenates.

Endogenous NA (µg)	NA added (µg)	Total NA (µg)		% Recovery of total NA
		Calculated	Found	
8.8	1.5	9.6	10.3	107
7.4	5	15.5	12.4	80
7.1	10	17.1	11.4	67

The mean value from 3 replicates was 7.76 µg. Since each moth
weighs approximately 100 mg and 15 moths were used for each
estimation, this gave an approximate value of 5.2 µg/gm of
insect. It was noted that the extraction efficiency
decreased with increased loading of noradrenaline standard to
aliquots of moth homogenate.

The procedure might be improved in future experiments by
increasing the value of butanol. From these preliminary
experiments it was concluded that noradrenaline was a major
component of the total catecholamines present in _Diparopsis_,
while adrenaline was present in extremely small amounts.

DISCUSSION

1. The possible influence of endogenous biogenic amines on insect reproductive processes.

Sub-lethal topically applied doses of the carbamate insecticide carbaryl and injection of three s-triazine chemosterilants to female Diparopsis enhanced the rate of oviposition without reducing fertility. Treatment of male moths with carbaryl and a series of s-triazines generally caused an increase in mating disfunction, expressed either as an absence of sperm from the spermatheca, permanent copulation or spontaneous extrusion of a spermatophore. Higher and lethal doses of carbaryl also consistently induced in the male before death the extrusion of a spermatophore. This was not merely due to stress as the result of poisoning, since other insectitides tested including DDT, diazinon, dichlorvis, dieldrin, BHC, menazon and pyrethrins failed to cause such an effect. Comparable results were obtained with another carbamate physostigmine.

In contrast, the s-triazine hemel, applied to male houseflies showed weak sterilising activity and caused a marked reduction in oviposition when they were mated to untreated females; while direct treatment of female flies inhibited oviposition completely (Borkovec & Terry, 1965). Similarly when hemel was applied to the diet of the olive fly Dacus oleae; 50% sterility occurred

in the female insect with reduced oviposition, while a
reduction of 90% in the rate of oviposition was noted when
male insects were treated and mated with untreated females
(Orphanidis& Patsakos, 1969). A reduction in fecundity also
occurred after oral treatment with certain s-triazines of the
pea-aphid Acyrthosiphon pisum Harris (Bhalla & Robinson, 1968).
Sub-lethal doses of carbaryl and other carbamate insecticides
also markedly reduced fecundity when applied to female houseflies
either before or after mating (Georghiou, 1965).

Thus certain s-triazines and carbamate insecticides
reduced fecundity in houseflies, whereas in the Lepidopteran
Diparopsis, similar treatment caused an increase in oviposition
in female moths and increased mating disfunction in male moths;
perhaps the result of hyperactivity of the accessory glands.
Where normal matings occurred there was no evidence to
suggest that sterility had been induced directly in the sperm
or egg.

No satisfactory explanation for the mode of action of
the melamine group of s-triazine chemosterilants has yet been
established. Hydroxymethyl derivatives readily liberate
formaldehyde, a mild alkylating and mutagenic agent and its
liberation 'in situ' might account for the activity of hemel
and other melamines. Certainly a hydroxymethyl compound
showed slight sterilising activity against male Diparopsis
(Part I). Some s-triazines function as antimetabolites of

pyrimidines (Baker & Ho, 1965), and the pyrimidine antimetabolite 5-fluorouracil reduced fecundity in female Diparopsis (Part I). However no melamine derivative has yet been found to function as an antimetabolite in any organism (Borkovec & DeMilo, 1967).

It is suggested that chemosterilant activity as the result of treatment may be mediated through the varied release of endogenous pharmacologically active substances. The biologically active amine serotonin was not detected in untreated Diparopsis moths, although substantial levels were found in adult Dysdercus. Serotonin was also detected in the housefly, confirming the results of Hays et al (1969) although in the present study, none was found in adult male Tenebrio. Hinks (1967) failed to find the precursor of serotonin, tryptophan in the neurosecretory cells of a short-lived noctuid moth Philosamia cynthiaricini, although it was present in the longer living, adult feeding Noctua pronuba. There may therefore be a relationship between adult longevity and feeding habit to the presence or absence of unbound serotonin. However for whatever reason, the serotonin levels are seen to vary widely from one insect species to another.

Oral application of serotonin to houseflies and Mexican fruit flies caused a complete suppression of oviposition (Hays & Amerson, 1967; Benschotter, 1966). It is also of significance that mating difficulties in male houseflies occurred after oral treatment of serotonin (Wicht & Hays, 1967);

which was attributed to a lack of seminal fluid. Another
derivative of tryptophan melatonin reduced fecundity in the
boll-weevil (Moore & Taft, 1969). Oral treatment of houseflies
with reserpine was shown to cause the release of endogenous
serotonin while oviposition was again reduced (Hays et al, 1969).
Traces of serotonin were detected in female Diparopsis after
injection of reserpine, an effect also associated with a slight
reduction in oviposition and appreciable sterility. It is
possible that the inhibition of oviposition in houseflies and
other Diptera after application of certain s-triazine chemoster-
ilants and sub-lethal doses of insecticides is also related to
similar release of endogenous pharmacologically active
substances. This idea recieves some support from Butygin &
Vyatchannikov (1969) who showed that sub-lethal peroral
doses of carbaryl applied to rats, caused a marked increase in
serotonin found in the blood.

Treatment of Diparopsis with either carbaryl or hemel
however failed to cause the release of serotonin although
when measured spectrophotofluorometrically a marked increase
in catecholamines was detected. A major component of these
catecholamines was found to be noradrenaline. Catecholamines
certainly occur regularly in insect tissues (Ostlund, 1954;
Bjorklung et al, 1970).

According to Haggendal (1963) the principal pathway for
noradrenaline metabolism in the mammalian central nervous system
is by deamination, since monoamine oxidase inhibitors caused an

increase in the level of this catecholamine. It is therefore
possible that carbaryl and hemel are acting as monoamine
oxidase inhibitors in Diparopsis. Certainly one carbamate
compound, n-cyclopropylcarbamate has indeed been shown to be
a potent monoamine oxidase inhibitor in mammals (Schittler, 1967).

An increase in catecholamines in Diparopsis as a result
of treatment with carbaryl or hemel unaccompanied by the release
of endogenous serotonin may therefore stimulate excitatory
activity resulting in increased oviposition in females and
mating aberrations in male moths.

In other insect species, as in certain Diptera, the
predominating effect due to the release of serotonin is in
contrast a suppression of oviposition in females and reduced
seminal secretions in the male insects.

Selective action in different sites in the insect due to
the transport characteristics of the applied chemicals may also
be an important factor in determining which effect predominates.
For example homogenates of corpora cardiaca, (now suspected
to contain serotonin) from Periplaneta americana and Blaberus
cranifer injected into the abdomens of untreated cockroaches,
caused decreased muscle tonus and quiescence(Ozbas & Hodgeson,
1958). Similar homogenates injected into the head capsule,
increased efferent activity, which was interpreted as the
suppression of the inhibitory influence of the sub-oesophageal
ganglion (Milburn et al, 1960).

2. Biogenic amines and endocrine misfunction.

The induction of biogenic amines as the result of chemosterilant treatment also has much wider implications. According to Berkoff (1969) substances such as noradrenaline exert brain hormone like activity in insects, a property also noted for the juvenile hormone analogues farnesol, farnesyl methyl ether and farnesyldiethylamine. Related substances also caused increased oviposition in Dysdercus (Carlisle & Ellis, 1967). Brain hormone (AH) according to Novak (1966) stimulates the prothoracic glands to release moulting hormone (MH) and also activates the corpora allata to produce juvenile hormone (JH).

The stimulation or suppression of an insect hormone at the wrong time in its life-cycle by pharmacologically active substances may be another way of manipulating insect growth and reproductive processes in the services of pest control. This speculation is given some credance in that an imidazoline compound, belonging to a group of chemicals known for their adrenergic blocking activities in mammals acted as a growth inhibitor and chemosterilant in several insect species (Schaeffer & Tieman, 1967). While of especial interest was the observation of these workers that treated corn-ear worm larvae Heliothis zea normally died during moulting from one instar to the next. In other words the normal moulting process was suppressed.

The effect of certain s-triazine chemosterilants against houseflies may therefore be twofold. In female insects, the release of endogenous serotonin would suppress oviposition. The stimulation of catecholamines may at the same time exert brain hormone like activity resulting in the release of abnormal levels of juvenile hormone from the corpus allatum. Reduced levels of juvenile hormone would lead to a degeneration of follicle cells and a resorption of mature eggs. Such effects were noted after corpus allatum extirpation in Rhodnius (Wigglesworth, 1936) and Calliphora (Possompès, 1955); and after treatment of Phyrrhocoris with an antimetabolite, 6-azauridine, which caused a reduction in corpus allatum size (Masner & Mácha, 1968). On the other hand hypersecretion of juvenile hormone could lead to abnormal yolk formation in young eggs and a distortion of subsequent embryological development of mature eggs typically found after application of certain juvenile hormone analogues. It is of interest to the idea of increased hormone secretion that Landa (1969) claimed that the sterilising effects of certain s-triazines in the housefly were due to an acceleration of the rate of embryological development.

Riddiford & Williams (1967) showed that DMF treatment of eggs of the silkmoths Hyalophora cecropia and Antheraea pernyi oviposited at least 5 hours previously, hatched normally, although suppression of 3rd or 4th instars occurred. Application

of other J.H. analogues including juvabione   to eggs of the
bugs Pyrrhocoris and Oncopeltus caused an even greater delayed
effect in that the final metamorphosis to the adult stage 4 weeks
later was suppressed (Riddiford, 1970). Such a delayed effect
may also have occurred in Diparopsis where only minimal
suppression of egg-hatch was noted after the treatment of
female moths with a series of J.H. analogues (Part I), but
when no observations on the fate of subsequent larval
development was followed.

Similarly oral treatment of female houseflies with certain
s-triazine chemosterilants failed to prevent egg-hatch, although
subsequent pupal development was suppressed. This was
particularly noted for melamines with two free amino groups
(LaBrecque et al, 1968). Such compounds produced a substantial
level of mating disfunction in male Diparopsis (Table 45).

The effect of juvenile hormone secretion in adult male
insects is not yet well established. Wigglesworth (1964) has
suggested that it may be related to accessory gland secretion;
particularly spermatophore production in male moths. A
reduction in the seminal fluid of houseflies was noted following
serotonin treatment (Wicht & Hays, 1967) while the reduction
in oviposition in olive flies when mated to hemel treated males
(Orphandis & Patsakos, 1969) may also be for the same reason.

3. Endocrine misfunction and effects on sperm.

Endocrine misfunction might also directly cause sterility of sperm in some species of insect. For example, males of many Lepidoptera can produce two kinds of sperm; functional eupyrene and non-functional apyrene sperm (see review by Virkki, 1963). An endocrinological control of this dimorphism was suggested from the work of Machida (1929), who transplanted Bombyx testes from pupae to larvae and vice versa and showed that the factor responsible for apyrene was in the pupal haemolymph. The occurrance of two kinds of sperm was not observed in Diparopsis, although some degenerate forms were noted in Autographa, where unlike Diparopsis active spermato-genesis was still occurring in the adult moth.

The only other group of insects where a similar anomaly has been reported with some regularity is the pentatomid group of Hemiptera where it is limited to one lobe, the "harlequin lobe" of the testes (Schrader, 1960). Induction of increased apyrene sperm in larval of pupal stages of lepidopterous pests might be a highly selective way of inducing sterility in this group of insects. According to Mann (1964) several species of vertebrate spermatozoa differ from other cells in their reduced ability to decompose hydrogen peroxide. The lack of catalase in mammalian semen therefore explains the particularly harmful effects of hydrogen peroxide and pure oxygen on spermatozoa. Furthermore spermatozoa can themselves produce

hydrogen peroxide 'in vitro' during oxidation of certain amino acids, hence any process enhancing such metabolic activity might lead to sperm degeneration. Juvenile hormone, activated by brain hormone is certainly a factor governing metabolism, consumption of food reserves in fat-body and increased oxygen consumption (Novák, 1966). The sterilising effects of some chemosterilants mediated through the release of biogenic amines might therefore involve the induction of apyrene sperm which would simulate the effects of direct mutagenic action.

4. The possible effects of aziridines and
   antimetabolites on endocrine misfunction.

Similar mechanisms may also be a factor involved in the sterilising effects of aziridine chemosterilants. A reduction in the volume of the corpora allata was certainly noted following apholate treatment of Phyllognathus silenus F. (Gruner, 1968) Earlier, reference was also made to the work of Masner & Mácha (1968) who showed that the antimetabolite 6-azauridine applied to newly emerged female Pyrrhocoris interfered with corpus allatum function. The resorption of eggs noted in Diparopsis (Part IV) and in other noctuids (Bulyginskaya et al, 1967) following tepa treatment may similarly be associated with endocrine misfunction.

Derivatives of chloroethylamine related to nitrogen mustards are in fact specific antagonists of adrenaline in mammals (Wilson & Schild, 1968).

5. Endocrine misfunction and mating behaviour

after chemosterilant treatment.

Houseflies sterilised with certain aziridine chemosterilants were reported to be more sexually vigorous than normal males LaBrecque et al, 1962). A similar effect was observed by Dame et al (1964) while studying the field behaviour of tepa-treated Anopheles quadrimaculatus, and by Gangrade & Pant (1970) after apholate treatment of Cadra cautella. Such hyperactivity may again be related to the increased release of endogenous biogenic amines such as noradrenaline. No convincing evidence for increased sexual vigour was found for male Diparopsis after extensive competitive mating tests (Part I & II). However when the mating frequency of treated males was expressed as a percentage of the control mating, clear hyperactivity was seen following treatment of males with sub-lethal doses of carbaryl and females with 2-4-diamino-6-morpholino-s-triazine HCl (Part I).

These speculations may be of value in suggesting future lines of work.

CONCLUSIONS

It is concluded that the administration of a chemosterilant may induce one or several effects on the reproductive system of an insect. The effect predominating will depend not only on the nature of the chemical but also on the stage, sex and condition of the gonads at the time of treatment. Where insects are sexually mature on adult emergence as occurs with Diparopsis then the primary effect may well be a direct action on the spermatozoa or eggs which suppresses or hinders subsequent embryological development. In the case of sperm, partial or complete immobolisation would prevent fertilisation. Where active gametogenesis is in progress at the time of treatment, rapid damage to the dividing cells may occur resulting in the reduced necrotic ovaries and testes observed in a variety of insects after application of aziridine and antimetabolite chemosterilants. In the case of Diparopsis only the young developing oocytes were therefore damaged as the result of treatment. Secondary effects may also occur as the result of changes in endocrine function either following the direct action of the chemosterilant on the hormone secreting organs or also possibly through the induced release of endogenous pharmacologically active substances. These secondary effects are exemplified by the resorption of mature eggs, a suppression of later larval or even adult stages after

apparently normal initial embryological development in the
egg and aberrant mating behaviour. The varied stimulation of
the endocrine systems of different insect species, with
sterilisation being a manifestation of such induced changes
in hormone secretions, may explain the high degree of specificity
so often noted for the non-alkylating chemosterilants and in
particular the s-triazines.

In the meantime despite the many chemicals that have
been shown to exert some effect on insect reproductive
processes, the only really practical chemosterilants as pest
control agents remain the aziridines.

The mutagenic hazards of this group of chemicals precludes
their wide-spread usage against the natural insect population,
while for _Diparopsis_ at least, the combination of a sex
attractant and chemosterilant in an autosterilising bait station
does not seem particularly promising. However as a substitute
for radiation for the sterilisation of certain species of mass
reared or mass collected Lepidoptera the aziridines offer
distinct advantages. Sterility can be rapidly induced in adult
insects by contact or oral methods of application, and no
elaborate sterilising apparatus is therefore required; a
necessary pre-requisite for insects like _Diparopsis_ where
suitable field experiments may be required in remoter parts
of Central Africa. Furthermore unlike radiation treatment of
Lepidoptera, there is virtually no deleterious effects on the

mating vigour and longevity of the sterilised male moths. The degradation studies of one aziridine, tepa in male _Diparopsis_ showed that the rapid metabolism of the chemosterilant under temperature conditions prevailing in Central Africa would ensure a minimal contamination of the environment following the release of tepa sterilised insects.

The release of sterile male _Diparopsis_ may be of value as part of an integrated control programme aimed at the whole cotton pest complex. Such an objectife is at present being considered by the Agricultural Research Council of Malawi. However to have any chance of success, the natural population of _Diparopsis_ in any particular area would first have to be reduced to a low level either by the application of insecticides or perhaps by the intensive trapping of male moths using the synthetic pheromone expected to be shortly available. Another possible use of sterilised insects would be to contain the spread of _Diparopsis_ from its existing territory.

Diparopsis castanea and the closely related _Diparopsis watersi_, although prevalent in most cotton growing areas of Africa are at present not found in East Africa. According to Pearson (1958) the primary reason for the failure of _Diparopsis_ to colonise these important equatorial regions is lack of opportunity, since throughout the interior of Africa in these latitudes there are no suitable wild host-plants, _Diparopsis castanea_ has long been established in Southern and parts of

Central Africa; moreover without adequate control of this pest high economic yields of cotton are impossible (Tunstall & Matthews, 1965).

No doubt as the result of increased cotton production, Diparopsis has recently appeared for the first time in Zambia (M.J.Way, private communication) and also Southern Tanzania (C.B.Cottrell, private communication). This suggests that its continued northern spread into East Africa is almost certain. Where low numbers of insects are entering new territory and where the use of insecticides to control such small numbers would therefore be uneconomic, the release of tepa sterilised male moths could be used to provide an effective control barrier to limit the further spread of such an economically important pest.

SUMMARY

1. A primary evaluation of 44 chemosterilants, comprising aziridines, a methanesulphonate, phosphoramides, s-triazines, antimetabolites, organo-metals, juvenile hormone analogues, miscellaneous agents and an insecticide was carried out using an injection method of application to a noctuid moth Diparopsis castanea Hmps. Only the aziridines exhibited pronounced chemosterilant activity. Weak sterilising action was shown by the phosphoramide hempa certain s-triazines and organo-tins, particularly against male moths. The antimetabolite cycloheximide sterilised female moths at a low dose of 0.1 μg but was also highly toxic. Slight sterilising activity against female moths was shown by the juvenile hormone analogue farnesyl methyl ether at a dose of 500 μg. Sub-lethal doses of the insecticide carbaryl caused a marked increase in the rate of oviposition.

2. A secondary evaluation of active chemosterilants against Diparopsis showed that the male sterility indices (LD_{50}/SD_{50}) for the aziridines apholate, tepa and metepa were 24, 17.9 and 11.5 respectively. Both hempa and triphenyl tin acetate had low sterility indices of 1.4 and -7.7. The sterility index of tepa for female Diparopsis was 4.2. Tepa treatment of female moths at doses causing complete sterility reduced mating effectiveness and also significantly reduced the oviposition rate.

Competitive mating tests of a duration of one night only showed that both apholate and tepa were equally effective in inducing sterility in male Diparopsis without loss of mating vigour. When males were repetitively mated to fresh virgin females on successive nights, tepa treatment at the SD_{95} level had no significantly adverse effect on the mating frequency. Apholate treated males at such a dose level however mated significantly less frequently. Tepa was therefore selected as the most effective chemosterilant.

The sterilising effect of tepa in male Diparopsis was permanent throughout a 6-day period, which is virtually the adult life span at $27^{o}C$. Topical treatment of male moths with tepa gave a sterility index of 34.6. At the SD_{95} level, tepa topically treated males were fully competitive.

Injected doses of 10 μg of tepa applied to adult male Autographa gamma L. induced 99% sterility in the eggs of untreated females to which they were mated. Unlike Diparopsis where normally the female mated only once, the Autographa females mated several times.

3. For the development of a chemosterilant bait-station as a possible control agent for Diparopsis it is desirable that attracted male moths should acquire an effective dose of chemosterilant either by brief contact with, or by momentary probing on, chemosterilant treated surfaces. It was estimated and later confirmed that thirsted moths probing on a 0.1% aqueous solution of tepa would take up the equivalent of a

sterilising dose, male moths so treated were consistantly sterilised with no adverse effects on mating frequency, longevity or competitiveness.

A prototype bait-station was used in the laboratory with virgin females as bait, which consistantly attracted male moths of various ages. The female moths were attractive for at least 8 days. A marker dye technique made it possible to determine whether the males would actively probe on arrival at the bait-station and so sterilise themselves. At temperatures of 25-27°C probing did not occur to an appreciable extent until the males were at least 3 days old, whether or not flight activity had previously occurred. Since both sexes of Diparopsis are sexually mature on emerging as adults it was concluded that the control method tested was unlikely to be effective. To facilitate chemosterilant penetration after momentary contact a series of vegetable oils were applied to male moths, but all reduced mating effectiveness. Male moths were however consistently sterilised by overnight contact on filter-paper saturated with 0.2% aqueous tepa. Repetitive mating tests also showed that the moths so treated were sexually competitive and equally responsive as untreated males to virgin females in the bait-station.

Population models constructed from data relevant to the biology of Diparopsis, indicated that the improvement in control by sterilising males attracted to the bait-station compared to

killing them would be slight at a 90% level and above but
would progressively increase at lower control levels.

4. A rapid rate of tepa degradation occurred in male Diparopsis
after injection application; half-life values ranging from
14.3 hours at 27°C to 18.6 hours at 15°C. After topical
application, tepa degradation occurred much more slowly
reaching the 50% level after 45.6 hours at 27°C, 72.3 hours
at 20°C and 143.3 hours at 15°C. The results were related to
the slow rate of absorption from acetone solutions. It was
concluded that at the high mean temperatures occurring in
Malawi where the insect is prevalent, the environmental hazard
from the release of tepa sterilised moths would be minimal.

5. Injected doses of tepa applied to female Diparopsis after an
interval of 3 days, caused a degeneration of the developing
oocytes and a shrinkage or resorption of the mature eggs.
No observable damage to the testes of either Diparopsis or
Autographa was observed after similar treatment. When tepa
treated male Autographa were mated with untreated females, it
was observed by histological examination that some of the eggs
oviposited and incubated for 3 days showed early arrested
development. In other instances greater development although
of an abnormal kind occurred. After treatment of Diparopsis
male moths with graduated injected doses of tepa, apholate and
metepa, it was similarly observed that considerable egg
development without subsequent hatch occurred at the low dosage
levels. A reduction in alkaline phosphatase activity in the

testes of _Diparopsis_ after tepa treatment of male moths occurred

at a dosage level which also caused agglutination of sperm

subsequently stored in the spermathecae of untreated mated

females. Both these phenomena suggested that a reduction

in sperm motility had occurred as the result of tepa treatment.

However no obvious damage to the ultra-structure of the

spermatozoa of _Diparopsis_ was observed after tepa treatment

of male moths at a similar dosage level.

6. Sub-lethal topically applied doses of the carbamate insecticide

carbaryl and injection of certain s-triazine chemosterilants

to female _Diparopsis_, enhanced the rate of oviposition without

reducing fertility. Treatment of male moths with carbaryl

and certain s-triazines often caused an increase in mating

disfunction expressed either as an absence of sperm from

the spermatheca after an apparently normal mating, permanent

copulation or a spontaneous extrusion of a spermatophore.

Spectrophotofluorometric analyses were carried out using

Diparopsis moth homogenates to see whether the release of

endogenous biogenic amines as a result of treatment might be

responsible for inducing the observed aberrations in the

reproductive processes. Approximate levels of serotonin were

also determined for adult _Dysdercus_, _Musca_ and _Tenebrio_.

Small amounts of the biogenic amine were detected in female

Diparopsis treated by injection with reserpine when examined

24 hours post-treatment. Similar treatment also caused reduced

oviposition and partial sterility. No unbound serotonin was

found in untreated _Diparopsis_, nor after treatment with either carbaryl or the s-triazine hemel. Instead a marked increase in catecholamines was noted and a major component found to be noradrenaline. It was suggested that such induced changes in the levels of biogenic amines as the result of treatment might in turn detrimentally influence hormone secretions responsible for the normal regulation of reproductive processes.

APPENDICES

<u>Appendix 1.</u> An example of the data and methods employed for
the calculation of the sterility and mortality
regression lines and the test for parallelism.

Chemosterilant tepa. Adult male <u>Diparopsis</u> treated by injection.
Probit analysis data.

(A) Sterility effect.

Dose (μg)	log dose x 10	No of mated females	Total eggs	Steriles eggs
2.00	1.30	13	1849	1820
1.00	1.00	11	1232	912
0.75	0.88	14	1848	1214
0.50	0.70	14	1904	644
0		12	1846	286

$\bar{y}$	$\bar{x}$	b	s.e.b.	x^2	df	m	s.e.m.
5.1447	0.8897	4.4685	0.140	30.67	2	0.8572	0.0173

(B) Mortality effect

Dose (μg)	log dose	No. treated	No. dead
80	1.90	20	20
40	1.60	20	19
20	1.30	20	17
10	1.00	20	11
5	0.70	20	5
C		20	4

$\bar{y}$	$\bar{x}$	b	s.e.b.	x^2	df	m	s.e.m.
5.4077	1.1918	3.3652	0.742	1.59	3	1.068	0.0686

(C) Parallelism of regression lines.

	df	Sum of squares	Mean square
Parallelism of regressions	1	1.05	1.05
Residual heterogeneiety	5	32.26	6.45
Total	6	33.31	

m_m	m_s	m_{rd}	$s.e.m._{rd}$	b	s.e.b.	h.f.
2.0997	0.8570	1.2427	0.1217	4.4303	0.1378	6.45

Appendix 2. Calculations for population models.

Designations.

V_i = number of virgin females emerging on day i
M_i = number of males emerging on day i
S_i = number of virgin females present in the population on day i
T_i = number of males in population on day i
R_i = number of fertile males in population on day i
F_i = number of females mating on day i
C_i = number of fertile females in population on day i
V_o = number of caged virgin females
P_i = probability of a male being attracted to a wild virgin female

I. No treatment.

Since it is assumed that all females emerging on day i will mate that day, the number of females mating on day i (F_i) is the same as the number of virgin females emerging on day i (V_i). Thus

$$F_i = V_i$$

The number of mated females on day i will be the sum of the females mating that day plus the surviving mated females from the previous day. Therefore the number of fertile females in the population on day i is

$$C_i = F_i + C_{i-1}d \quad \text{where d is the survival rate.}$$

If V_i = 15 and i any number from 1-120 and d = 0.75 then

Day 1 $F_1 = C_1 = 15 + C_1$?
               $F_1 + C_o (0.75) = 15 + (0 \times 0.75) = 15$

Day 2 $F_2 = V_2 = 15 + C_2$
               $F_2 + C_1 (0.75) = 15 + 15 \times 0.75 = 26$

Day 3 $F_3 = V_3 = 15 + C_3$
               $F_3 = C_2 (0.75) = 15 \times 26 \times 0.75 = 35$ and so on.

II. The emerging population in the 1 acre isolated area is exposed to a total of 375 or 100 or 50 caged virgin females (i.e. ratios of 25:1, 6.67:1 and 3.33:1 on a daily emergence basis), which compete fully with wild females in attracting males. The number of virgin females in the cages will be V_o. It is presumed that all males attracted to the cages will be killed immediately or trapped. Males that mate with wild virgin females will redistribute themselves in the population and on the following day those surviving natural hazards will again respond either to caged or wild virgin females.

Since the number of wild virgin females in the population on day i is the sum of the virgins emerging that day plus the survivors from previous day. Those present on day i

$$S_i = V_i + (S_{i-1} - F_{i-1}) d.$$

Also the probability of a male being attracted on day i to one of the wild virgin females is

$$P_i = S_i / (V_o + S_i).$$

Also the number of live fertile males in the population on day i will be the sum of the males emerging on day i (M_i) plus the survivors of the males that mated with wild virgin females (F_i) the previous day.

$$R_i = M_i + F_i - {}_1d$$

and the number of females mating on day i will be

$$F_i = P_i R_i$$

and the number of fertile females in the population on day i will be

$$C_i = F_i + C_i - {}_1d$$

III. Model as II but males are sterilised when coming in contact with caged virgins and returned to the population.

Then
$$S_i = M_i + T_i - {}_1d \qquad \text{and}$$
$$R_i = M_i + F_i - {}_1d .$$

Appendix 3. An example of the analysis of variance data used to test for the linearity of the regression lines in the tepa degradation studies.
15°C Injection application of tepa to male moths. Homogenization extraction.

log tepa recovered (µg/moth)	Rate of tepa degradation						Analysis of variance			
	Hours post-treatment						Nature of variation	d.f.	Sum of squares	Mean square
	0	3	6	12	24	48				
Replicates							Regression	1	1.1288	
	0.9294	0.9191	0.7709	0.7076	0.3979	0.3220	Deviation from regression	4	0.0054	0.0013
	0.9777	0.8129	0.8325	0.7243	0.5682	0				
	0.8672	0.8868	0.8325	0.6721	0.5911	0.2553	Between doses	5	1.1234	0.2246
sy	2.7743	2.6188	2.4359	2.1040	1.5572	0.5773	Within doses	12	0.0956	0.0079
n	3	3	3	3	3	3	Total	17	1.2190	
y	0.9247	0.8729	0.8119	0.7013	0.5190	0.1924				

$\bar{x}$	$\bar{y}$	Sxx	Sxy	Syy	sum of squares between doses	$\dfrac{S^2xy}{Sxx}$
15.5	0.6704	4883	-74.24	1.2190	1.1234	1.1288

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