EFFECTS OF CHEMOSTERILANTS AND GROWTH REGULATORS ON THE PEA APHID, Acyrthosiphon pisum (Harris), FED ON AN ARTIFICIAL DIET

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ABSTRACT

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EFFECTS OF CHEMOSTERILANTS AND GROWTH REGULATORS ON

THE PEA APHID, Acyrthosiphon pisum, (Harris),

FED ON AN ARTIFICIAL DIET

The purpose of this investigation was to demonstrate that a technique of rearing aphids on an artificial diet could be used to determine the effects of chemosterilants and growth regulators on the mortality and fecundity of the pea aphid, Acyrthosiphon pisum (Harris). The technique employed for rearing pea aphids was that of Auclair and Cartier (1963). Nymphs were fed on a chemically defined diet to which test chemicals were added, and progeny were counted after the nymphs became adults and began reproducing. All tests were conducted under controlled conditions.

In this study thirty chemosterilants and three growth regulators were tested. Of these, eight compounds induced permanent sterility. These compounds included five aziridines, tepa, apholate, P, P-bis (1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787), P, P-bis (1-aziridinyl) N-methylphosphinic amide (ENT-51,254), and P, P-bis (1-aziridinyl)-N isopropylphosphinic amide (ENT-51,256); two antimetabolites, methotrexate and 5-fluorouracil; one triazine derivative, 3,5-diamino-6-phenyl-1,2,4-triazine (ENT-60,279). Among these materials, tepa, apholate, metho-

trexate and 3,5-diamino-6-phenyl-1,2,4-triazine inhibited reproduction over a wide range of concentrations without apparent toxic effect.

Two compounds, 1-acetyl-2-thiourea (ENT-24,935) and an imidazole derivative (ENT-60,194) were found to be temporary sterilants. The sterility induced was reversed when treated females started feeding on an untreated diet.

Seventeen chemosterilants, including some known insect sterilants (metepa, hemel, and hempa), and three growth regulators, maleic hydrazide, cycocel, and "queen substance" significantly reduced fecundity. The decrease in fecundity was apparently due to the presence of test chemicals in the synthetic diet.

Three compounds, P, P-bis (1-aziridinyl) N, N-dimethylphosphinic amide (ENT-50,990), ENT-60,345 (s-triazine derivative) and thiohempa, were toxic at dosages tested.

Ovaries of females treated with effective chemosterilants showed marked reduction in size. The effect was more pronounced on the anterior of the ovarioles. Degeneration of inhibited ovaries became progressively more severe from anterior to posterior.

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CHAPTER I

INTRODUCTION

In recent years, the use of chemosterilants for controlling insect populations has received considerable attention. Many different chemicals have been found which affect the reproduction of various insects, and to date several insect species have been effectively sterilized by application of chemicals.

The problem

The present studies were intended to supplement the rapidly accumulating information on the induction of sterility in insects. Although extensive work has been conducted on insect chemosterilization, little work has been done with insect species of the Order Homoptera. Earlier, Harries and Mattson (1963) and Robinson (1959, 1960) studied the effect of certain chemicals on the mortality and reproduction of aphid species, but the techniques employed were not entirely satisfactory. In their techniques, there was no way of knowing in what dilution the material entered the body of the aphids. Recently, Auclair and Cartier (1963) developed an artificial method of rearing the pea aphid, Acyrthosiphon pisum (Harris), on a chemically defined diet. With this technique, measured quantities of chemicals, known to affect reproduction, can be added to the pea aphid food. In the studies reported here, thirty three compounds were investigated for their effects on the mortality and fecundity of the pea aphid.

Importance of the study

The present study is the first serious attempt to use chemosterilants on insects which suck plant sap. At present there appears to be no practical way in which this information, demonstrating inhibition of reproduction in the pea aphid with chemicals, can be used in the field. However, it has been shown that the technique of rearing aphids on a chemical diet can be successfully used to determine the effect of chemosterilants on the mortality and fecundity of the pea aphid. With the information available from these studies, it may be possible to treat plants in the field with chemosterilants, for aphid control.

CHAPTER II

REVIEW OF THE LITERATURE

Reviews dealing with chemosterilants have been presented by Borkovec (1964) and Smith et al (1964). Recently a book on the subject has been published by Borkovec (1966). The use of chemosterilants for controlling insect populations has received considerable attention since the successful eradication of the screw-worm fly, Cochliomyia hominivorax (Coquerel)in certain areas, through the release of adult males sterilized by gamma radiation to mate with flies of natural populations (Baumhover et al 1955; Knipling 1955).

Borkovec (1964) discussed briefly the principles and shortcomings of radiation sterilization. Knipling (1959, 1962) pointed out the advantage of chemosterilization over the use of an insecticide. For example, an insecticide that kills 90% of an insect population will reduce its reproductive potential to 10%, but a chemosterilant affecting 90% of a population will reduce this potential to one per cent. With only one per cent reproducing, the population would be greatly reduced and after 5 generations would, theoretically, be eradicated.

Sterilization with chemicals is not a new concept for insect control, since it was suggested as early as 1937 (Knipling 1960). The inhibition by chemicals of ovarian development, and their effect on reproduction, were first reported for Drosophila melanogaster (Meigen) (Goldsmith and Frank 1952), and later in the house fly, Musca domestica L.

(Mitlin et al 1957). Interest in the use of chemical sterilants for insect control has increased since LaBrecque (1961) induced sterility in both sexes of the house fly by feeding apholate, tepa, and aphamide. To date, inhibition of reproduction with chemosterilants has been reported in a number of insect species representing the orders of Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Borkovec 1964; Chamberlain 1962; Crystal 1963; Davis and Eddy 1966; Grosch 1963; Hair and Adkins 1964; Harris 1962; Harris and Frazar 1966; Hedin et al 1964; Henneberry et al 1964; Howland et al 1965; LaBrecque 1961; Ladd 1966; Lindquist et al 1964; Shaw and Riviello 1962; Weidhaas et al 1962).

Study of the literature reveals that little is known about the effects of chemosterilants on haustellate insects. Harries and Mattson (1963) tested various antibiotics as sprays, for toxicity and sterilizing effects on three species of aphids. The possible sterilant effects of the more active antibiotics were not evident because of their toxicity, and those with lower or no toxicity did not show any marked sterilant effects although the number of progeny was generally less than in the treated control. Harries (1963) studied the effects of some antibiotics and other compounds on fertility and mortality of orchard mites. He found that the oviposition of the two-spotted spider mite, Tetranychus telarius (L.) and of the European red mite, Panonychus ulmi (Koch) was inhibited by a number of antibiotics. Smith et al (1965) treated two species of greenhouse spider mites with apholate dips, sprays, or residues. They reported that untreated females of the two-spotted spider mite, which mated with males dipped in 0.5% apholate in alcohol-water

solution, produced male progeny. Females exposed to 2% apholate dip produced no viable eggs.

Two groups of chemical compounds, antimetabolites and alkylating agents, have shown the most promise as insect sterilants. Antimetabolites and alkylating agents have a different mechanism for inducing sterility. Antimetabolites are compounds wherein a metabolite essential to cell development has been changed in one of several minor ways, and which when introduced into the animal, will elicit signs associated with a specific lack of the metabolite (Woolley 1952; Shive and Skinner 1958). An antimetabolite also may be designated a "metabolic antagonist" or a "substrate analogue". Antimetabolites such as the metabolic antagonists of amino acids, folic acid, purine and pyrimidine have been tested as insect sterilants. Antimetabolites such as aminopterin, amethopterin, 5-fluorouracil, and 5-fluoroorotic acid showed promise as insect sterilants, but sterilized females only (Crystal 1963; Goldsmith and Frank 1952; LaBrecque and Gouck 1963; LaBrecque et al 1960; Mitlin et al 1957; Painter and Kilgore 1964, 1965; Piquett and Keller (1962).

The alkylating agents commonly referred to as radiomimetic compounds replace hydrogen with an alkyl group in fundamental genetic material with an effect similar to irradiation (Alexander 1960). The alkylating agents are highly effective in producing mitotic disturbances or nucleotoxic conditions particularly in tissues where cell multiplication takes place at a high rate. Chemosterilants belonging to this group are nitrogen mustards and ethylenimine derivatives (aziridines). Of the

alkylating agents, aziridines have proved particularly useful because of their effectiveness in sterilizing both sexes of insects and their often wide toxicity-sterility margin (Borkovec 1964; Borkovec and Woods 1963; Hair and Adkins 1964; LaBrecque 1961). However, there may be exceptions to the above mentioned generalization, depending upon the test insect and the mode of administration. Some of the most widely used representatives of this group are tepa, metepa, tretamine, aphamide, and apholate.

Lately, large numbers of candidate chemosterilants have been tested for their activity as insect sterilants (Fye et al 1965, 1966; Geering et al 1965; Gouck and LaBrecque 1964; Kenaga 1965; Ristich et al 1965; Woods et al 1964).

Many research workers have evaluated the effects of plant and animal growth regulators on the fecundity, growth and development of insects. Mitlin and Baroody (1958) tested fifteen synthetics and three materials of biological origin for their effect on ovarian growth, by feeding them to the house fly. They reported that coumarin, 1-phenyl-2-thiourea, piperonyl butoxide, p-quinone, and thiourea completely inhibited ovarian growth at concentrations of 5,5,1,5, and 2 mg./litre, respectively. Chamberlain and Hopkins (1960) observed reduction in size of ovaries of 4- and 8-day old house flies, obtained from larvae treated with 0.0002% colchicine. Similar results were reported by Mitlin et al (1957).

"Queen substance" chemically identified as 9-oxodec-trans-2-enoic acid (Butler et al 1961) inhibited ovarian development in the termite,

Kalotermes flavicollis (F.) (Hrd'y et al 1960) and in house flies (Nayar 1963). Contrary to these observations, Mitlin and Baroody (1958) failed to obtain inhibition of ovarian development in house flies by feeding or injection of queen bee extract. Pain (1961) obtained no inhibition of ovary development in worker honey bees by synthetic queen substance alone.

Biologically active growth regulators have been found to have some or no sterilizing activity by many workers (Benschoter 1966; Edel'man and Efros 1962; Emden 1964; Keiser et al 1965; Robinson 1959, 1960; Simkover 1964; Tahori et al 1965; Yule et al 1966).

For biological evaluations, the mode of administration of a chemosterilant varies quite widely according to the insect species and the compound employed. Larvae, pupae, and adults may be sterilized by chemical treatment. The egg seems to be the only stage on which chemosterilants have either no effect, or function as ovicides. A technique found convenient for use in screening programmes is to measure the fecundity and fertility of adult insects reared on a diet in which a specific amount of chemosterilant has been incorporated. The candidate chemosterilants have been administered to house flies, in sugar or other dry food (Gouck et al 1963a; LaBrecque 1961), to screw-worm flies in sugar solution (Crystal 1963), to tephritid flies in a mixture of granulated sugar or orange crystals supplemented with protein hydrolysate (Shaw and Riviello 1962; Keiser et al 1965), to plum curculios (Hays and Cochran 1964) and to boll weevils (Hedin et al 1964) in partially artificial diets. Other methods of administration of chemosterilants are available, for

example, injection (Borkovec et al 1964; Chang 1965a; Chang and Borkovec 1964; Chang and Kearns 1962), topical application (Chamberlain and Hamilton 1964; Crystal 1964b; Ouye et al 1965; Parish and Arthur 1965), contact with treated surfaces (Collier and Downey 1965; Dame and Schmidt 1964; Harris 1962; Howland et al 1965; Keiser et al 1965), dipping (Henneberry et al 1964; Lindquist et al 1964; Roach and Buxton 1965), dusting and spraying (Chamberlain 1962).

Pupae, because of their inactivity, are more easily handled than larvae or adults. Bushland and Hopkins (1953) found that the screw-worm fly was most susceptible to sterilization by gamma radiation while in the pupal stage, and pupal irradiation has been used with great success in programmes involving the weekly sterilization and release of millions of flies of this species (Baumhover et al 1955; Lindquist 1955).

Chamberlain (1962) achieved sterility by dipping prepupae of the screw-worm fly in apholate, but pupal treatment resulted in only partial sterility. Piquett and Keller (1962) reported that when puparia containing pupae about 4 days old were immersed for 30 minutes in equal parts of acetone and water saturated with a known chemosterilant (2,2'-dichloro-N-methyldiethyl amine) oviposition was prevented in the emerging house flies. Gouck (1964) induced sterility in house flies by dipping puparia containing pupae of different ages in apholate, tepa, and metepa at concentrations of 2.5 and 5% for 30 to 300 seconds. At all dipping periods and with concentrations at 2.5 and 5%, apholate and metepa gave most consistent sterility in flies emerging from puparia dipped when pupae were 2 days old and tepa in flies from puparia dipped

when pupae were 1 day old. Induction of sterility by treating pupae of the face fly, Musca autumnalis DeGeer, was demonstrated by Hair and Adkins (1964).

Shaw and Riviello (1965) found that the Mexican fruit fly, Anastrepha ludens (Loew), could be sterilized by dipping pupae for 60 seconds in a 5% solution of tepa in methanol, provided that the adults were allowed to emerge from the treated puparia, but not when the puparia were washed and drained. Keiser et al (1965) also obtained sterilization of fruit flies by dipping pupae in a chemosterilant solution, one day before the emergence of fruit flies.

Contrary to these findings, pupal treatment with chemosterilants at sterilizing dosages proved ineffective against the gypsy moth, Porthetria dispar (L.) (Collier and Downey 1965) and the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Young and Cox 1965). In general, the age of the pupae was a critical factor and sometimes a higher mortality occurred than was desired.

Induction of sterility by treating the larval stage might be advantageous with some insect species, particularly if the insects could be reared and sterilized in large numbers for release. Weidhaas (1962) observed complete to partial sterility when mosquito larvae of Aedes aegypti (L.) were reared in water treated at 10 p.p.m. of apholate. Dame and Schmidt (1964) obtained only 36 per cent reduction in fertility of mosquitoes with metepa at 10 p.p.m. in the larval medium. Chamberlain (1962) obtained greatly reduced fertility in screw-worm flies when larvae were reared in media containing 25 to 50 p. p. m. of apholate but

treatments had deleterious side effects. Gouck et al (1963a) reported more toxicity than sterility when larvae of the house fly were dipped in chemosterilant solutions. Similar findings were made by Shaw and Riviello (1962) in larval applications to fruit flies.

The treatment of insects with chemosterilants in the larval stage seems impracticable not because sterilization cannot be effected but because of the concurrent high mortality. In addition, the adults which survive chemical treatment remain small, and it is questionable whether they could successfully compete with wild males for females.

There is no doubt that insects can be sterilized by chemical means but there is considerable species specificity. Response of a particular species could be as important with chemosterilants as with insecticides. Apholate was more effective as a sterilant of stable flies than of screw-worm flies when both sexes were treated topically. Chamberlain (1962) reported that screw-worm flies could be sterilized at 150 µg per fly, or about 3,045 µg per gram of body weight. Stable flies were sterilized at 1.0 µg per fly, or about 67 µg per gram of body weight (Harris 1962). Therefore, screw-worm flies required 45 times more apholate for sterilization than stable flies. Chamberlain and Barrett (1964) studied the differential susceptibility of males and females to metepa, with topical treatments. The male screw-worm fly required 5.5 times as much metepa per gram of body weight as the male stable fly, and the female screw-worm fly required 18 times as much as the female stable fly. The values for feeding treatments of the screw-worm fly and stable fly were 3.9 and 6.2 times, respectively, for male and female.

Keiser et al (1965) demonstrated that the male melon fly, Dacus cucurbitae Coquillett was the most susceptible to chemosterilants; the oriental fruit fly, D. dorsalis Hendel, intermediate; and the Mediterranean fruit fly, Ceratitis capitata (Wiedemann)the least susceptible.

The mode of treatment also has a considerable bearing on the degree of sterilization desired and the mortality which could be tolerated. Chemosterilants are generally more effective orally than topically. Crystal (1966) examined the results of all aziridinyl compounds tested against the screw-worm fly. He concluded that more compounds were potent chemosterilants (90%) and non-toxic (38%) when administered multiple-orally than topically (44% and 20% respectively). He further indicated that toxicity of compounds was largely unrelated to functionality, to substitution, and only partly related to mode of administration. However, he found that aziridinyl compounds do differ widely in their toxicity to screw-worm flies (Crystal 1963, 1964b,c). He suggested that these differences are most likely due to substitution on the aziridinyl nitrogen, the carrier portion of the molecule.

The translocation and fate of chemosterilants in insects and mammals is, of course, of the greatest importance. The metabolism of the alkylating agents by larger animals has been studied to a considerable extent, and work conducted before 1958 was reviewed by Smith et al (1958). Chamberlain and Hamilton (1964) studied the rate of absorption, excretion, and metabolism of P^{32}-labeled metepa by the screw-worm fly and the stable fly to explain the difference between the dosages required to sterilize these two species. They found that the screw-worm fly

absorbed only half as much radiolabeled material in proportion to its size as the stable fly, and the excretion was twice that of the stable fly, and metabolism by the stable fly was about twice as fast as for the screw-worm fly. Plapp et al (1962) investigated the fate of P^{32}-labeled metepa in mosquitoes, Culex tarsalis Coquillett; house flies, M. domestica L. and mice. Dame and Schmidt (1964) did additional research with mosquitoes, Anopheles quadrimaculatus Say and Aedes aegypti (L.). Parish and Arthur (1965) reported metabolism of P^{32}-labeled thiotepa in rats and four species of insects.

It is most important that the treated males remain in all biological functions fully competitive with untreated or natural insects. Chemically sterilized males of Aedes aegypti (L.) were shown to be as competitive in mating as untreated males (Weidhaas and Schmidt 1963). LaBrecque et al (1962a) found no impairment in sexual competitiveness of sterile males of the house fly. Rather, the treated males were more competitive sexually than the untreated males. Similar results were reported on the sexual aggressiveness of males in other insect species (Hays and Cochran 1964; Kido and Stafford 1966; Ouye et al 1965; Shaw and Riviello 1962; Young and Cox 1965). Henneberry et al (1966) indicated a decrease in mating efficiency of sterile males of the cabbage looper, Trichoplusia ni (Hubner).

The longevity of chemosterilized insects, particularly the males, has a significance in field applications. Some workers have indicated that sterilizing dosages do not affect male longevity (Howland et al 1966; Kido and Stafford 1966), while some noted reduction in the

longevity (Hedin et al 1964; Henneberry et al 1964). Murvosh et al (1964) found that although metepa substantially shortened the life span of house flies, 90% of the males survived the first 10 days, which time was sufficient to allow mating with most of the females that emerged at the same time as the males.

The chemosterilants produce dominant lethal mutations, or severely damage genetic material in the sperm and ova, thus ultimately causing reduction in the size of the reproductive organs. Cantwell and Henneberry (1963) by feeding one per cent apholate to adults of Drosophila melanogaster (Meigen) noted that a breakdown of the nurse cells, oocytes, and follicle cells occurred in the ovarioles of the treated females. Much the same effect was observed in house fly females given one per cent apholate in the food for periods up to 240 hours after eclosion (Morgan and LaBrecque 1962). Regardless of the stage of development the sterility induced was irreversible. LaChance and Crystal (1963) found that thiotepa on screw-worm flies was mutagenic to the anaphase 1 stage of meiosis in the oocyte, and the end result was death in the zygote owing to the failure of the completion of the meiotic division or chromosome loss or imbalance in the embryo. Dame and Ford (1964) suggested that chemosterilants applied to inseminated female mosquitoes may cause sterility by their effect on the stored sperm within females.

Generally, in the administration of effective dosages of chemosterilants to insects, there has been shown marked reduction in the size of the reproductive organs (Chamberlain 1962; Keiser et al 1965; Kido

and Stafford 1966; Lindquist et al 1964; Mitlin and Baroody 1958; Mitlin et al 1954, 1957; Morgan and LaBrecque 1964; Painter and Kilgore 1965; Shaw and Riviello 1962).

Field experiments have shown that even in localities where re-infestation is almost certain, chemosterilants may offer a good insect control method. Gouck et al (1963b) applied a corn meal bait containing 0.75% apholate on a dump at Pine Island, Florida, for the control of house flies. The treatment gave considerable reduction of fly population. In similar studies, LaBrecque et al (1962b, 1963) used aphoxide and metepa at 0.5% to evaluate the efficacy of chemosterilants to control house flies in the field. Marked reduction in the fly population and egg hatch was noted. Shaw and Riviello (1965) released 2.5 million sterile Mexican fruit flies, Anastrepha ludens (Loew) in a 10 acre mango grove in Morelos, Mexico, during 1962 and 1963. They obtained substantial protection of the major crop against fruit flies. Recently Davich et al (1965) demonstrated that the sterile-male release technique could be used to eliminate relatively small, isolated populations of the boll weevil, Anthonomus grandis Boheman.

CHAPTER III

MATERIALS AND METHODS

The insect species used in these experiments was the pea aphid, Acyrthosiphon pisum (Harris) (Homoptera: Aphididae). The pea aphid culture was maintained on broad bean plants, Vicia faba L., variety Windsor. To obtain young aphids for tests, adult apterous females were placed on broad bean plants 2-3 inches high for less than 24 hours and then removed. The progeny remaining on the plants were approximately the same age.

In these experiments, the effects of various chemicals on the mortality and fecundity of A. pisum were investigated. The chemicals investigated are listed in Table I. Most of the chemicals tested were obtained from Dr. A. B. Borkovec, In Charge Chemosterilant Investigations, Pesticide Chemicals Research Branch, United States Department of Agriculture, Beltsville, Maryland. The code numbers from this laboratory are listed under the heading ENT-No. The test chemicals, at various concentrations, were mixed in a chemical diet and fed to the pea aphid.

The feeding cage and chemically defined diet were similar to those of Auclair and Cartier (1963) with certain improvements kindly suggested by Dr. J. J. Cartier (Canada Agriculture Research Station, St. Jean, Quebec). The composition of the diet is given in Table II. Figure 1 shows a diagrammatic view of the component parts of the feeding cage (Figure 2). The diet was formulated as required, stored in air-tight containers and held at -28°C.

Aphids used in these tests were placed in feeding cages at early third instar, 10 aphids per cage, unless otherwise noted. The aphids fed on the diet through unstretched Parafilm "M"(R) (Figure 3). The membrane known as Parafilm "M"(R), made by Marathon, American Can Company, Neenah, Wisconsin, is available commercially. They were given access to the chemical diet containing the test chemical for varying number of days and then transferred to the untreated diets. The cages were opened after the nymphs had become adults. The numbers of nymphs surviving to adult stage in the different treatments were observed. The adults were then transferred individually to cages on untreated diets for larviposition. For the first seven days of the reproductive period, the feeding cages were opened daily in order to count live and dead larvae produced. The larvae after counting were removed from the cages.

All experiments were conducted in a plant-growth cabinet which maintained a temperature of 69°F., relative humidity of 70±2% and a photoperiod of 16 hours of light and 8 hours of darkness. A relative humidity higher than that of the growth cabinet was maintained by setting the feeding cages in water trays (Figure 4). Immediately above the feeding cages was orange lighting (595-600 mμ) with a light intensity at aphid level of 30-50 foot candle.

Analysis of variance was performed on the data on fecundity. Before the statistical analyses were conducted, the number of larvae produced per female was transformed by adding a common low value to each observation and taking the square root of the total ($\sqrt{n + 0.5}$) (Bartlett 1936). The t test was used to compare the difference between two

treatments (Snedecor 1961).

Effect on ovarian development

At all treatments, 1-2 extra feeding cages were maintained to determine the effect of the chemical on ovarian development. The adult females were dissected, under saline solution, to observe the development in these organs. The photographs of the ovary, placed on a drop of water, were taken by means of a 35 mm. Nikon camera mounted on a compound microscope.

TABLE I

LIST OF CHEMICALS TESTED

ENT-No.	Common Name	Chemical Name
24,915	Tepa	Tris(1-aziridinyl) phosphine oxide
26,316	Apholate	2,2,4,4,6,6-Hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine
50,003	Metepa	Tris(2-methyl-1-aziridinyl) phosphine oxide
50,787		P, P-bis(1-aziridinyl)-N-ethylphosphinic amide
50,990		P, P-bis(1-aziridinyl)-N, N-dimethylphosphinic amide
50,991		P-(1-aziridinyl)-N, N,N',N',-tetramethylphosphonic diamide
51,028		P, P-bis(1-aziridinyl)-N-butylphosphinic amide
51,253		P, P-bis(1-aziridinyl)-N-propylphosphinic amide
51,254		P, P-bis(1-aziridinyl)-N-methylphosphinic amide
51,256		P, P-bis(1-aziridinyl)-N-isopropylphosphinic amide
25,297		5-Fluorouracil
25,299	Methotrexate	N-[p-{[(2,4-diamino-6-pteridinyl)methyl] methylamino} benzoyl]-glutamic acid
50,852	Hemel	2,4,6-Tris(dimethylamino)-s-triazine
50,905		Hexamethylmelamine, hydrochloride
51,143		2,4-Diamino-6-morpholino-s-triazine, hydrochloride
51,454		s-Triazine derivative

(continued)

TABLE I (CONTINUED)

LIST OF CHEMICALS TESTED

ENT-No.	Common Name	Chemical Name
60,279		3,5-Diamino-6-phenyl-1,2,4-triazine
60,289		1,2,4-Triazine derivative
60,345		s-Triazine derivative
50,882	Hempa	Hexamethylphosphoric triamide
50,918	Thiohempa	Hexamethylthiophosphoric triamide
28,009		Triphenyl tin hydroxide
24,935		1-Acetyl-2-thiourea
60,194		Imidazole derivative
51,325		2-Chloroacetamidine hydrochloride
60,109		N-Oxide of a heterocyclic nitrogen compound
60,046		Ester of boric acid
51,068		1,1-(ethanediylidenedinitro) di-Guanidine, dihydrochloride
60,179		Thiadiazole derivative
60,302		Pyrimidine derivative
	Maleic hydrazide	1,2-Dihydro-3,6-pyridazindione
	Cycocel	2-Chloroethyl trimethylammonium chloride
	"Queen substance"	9-Oxodec-trans-2-enoic acid

TABLE II

COMPOSITION OF THE SYNTHETIC DIET

FED TO THE PEA APHID

1-Amino acids and amides (mg)	
Alanine	100
Arginine	400
Asparagine	300
Aspartic acid	100
Cysteine	50
Cystine	5
Gamma-amino butyric acid	20
Glutamic acid	200
Glutamine	600
Glycine	20
Histidine	200
DL-Homoserine	800
Isoleucine	200
Leucine	200
Lysine mono-HCl	200
Methionine	100
Phenylalanine	100
Proline	100
Serine	100
Threonine	200
Tryptophan	100
Tyrosine	20
Valine	200
	4315

(continued)

TABLE II (CONTINUED)

COMPOSITION OF THE SYNTHETIC DIET

FED TO THE PEA APHID

Vitamins (mg)	
Ascorbic acid	10.0
Biotin	0.1
Calcium pantothenate	5.0
Choline chloride	50.0
Folic acid	1.0
i-Inositol	50.0
Nicotinic acid	10.0
p-Aminobenzoic acid	10.0
Pyridoxine HCl	2.5
Riboflavin	5.0
Thiamine HCl	2.5
	146.1
Others	
Cholesterol benzoate	2.5 mg
K_3PO_4	500.0 mg
$MgCl_2 \cdot 6H_2O$	200.0 mg
Salt mixture no.2 U.S.P. XIII	5.0 mg
Sucrose	35.0 g
Water to make	100.0 ml

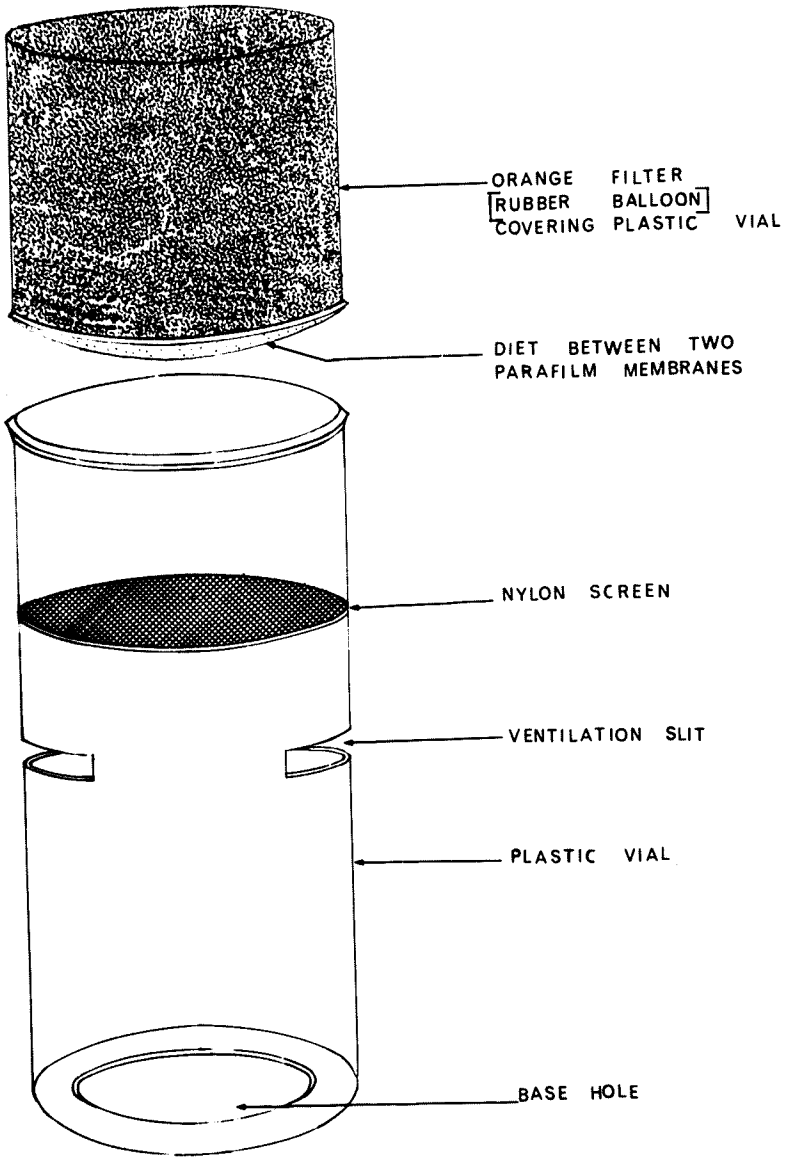

Fig.1. Diagrammatic view of the component parts of the aphid feeding cage.

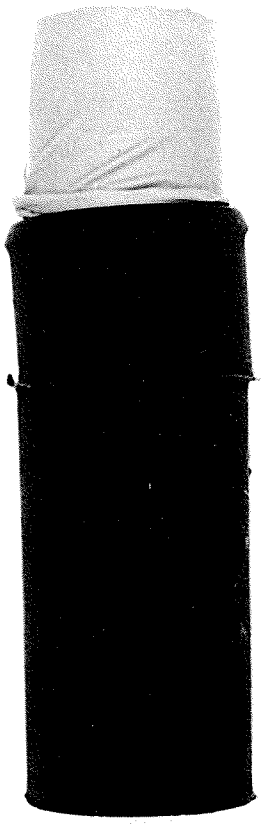

Fig. 2. Aphid feeding cage.

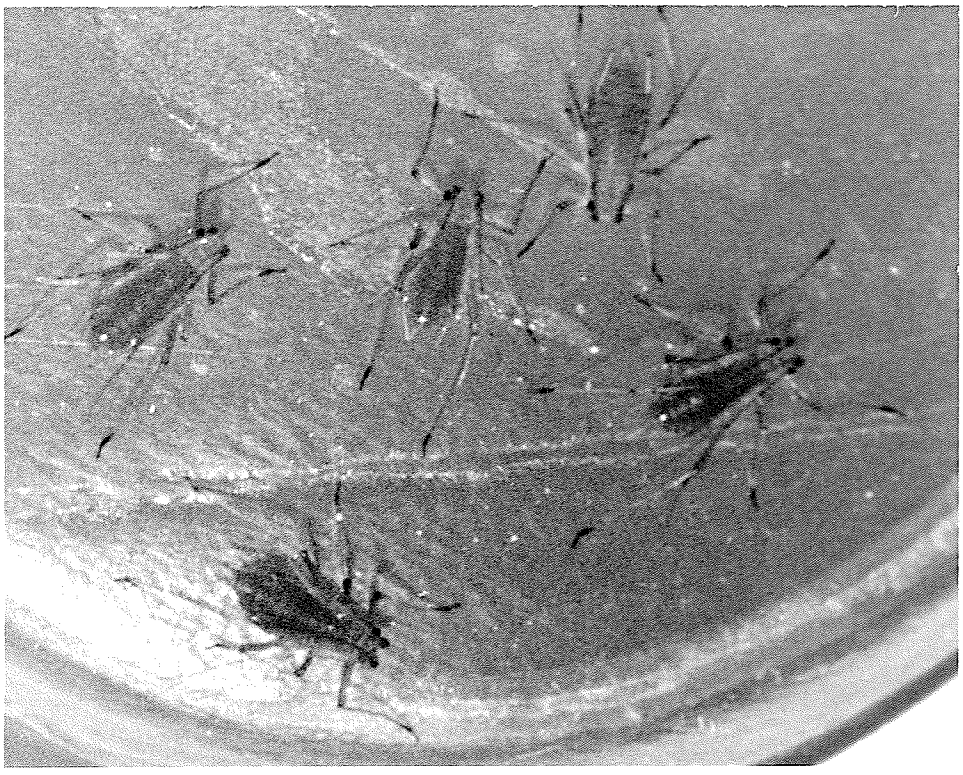

Fig. 3. Pea aphids feeding on a synthetic diet enclosed between two "Parafilm" membranes.

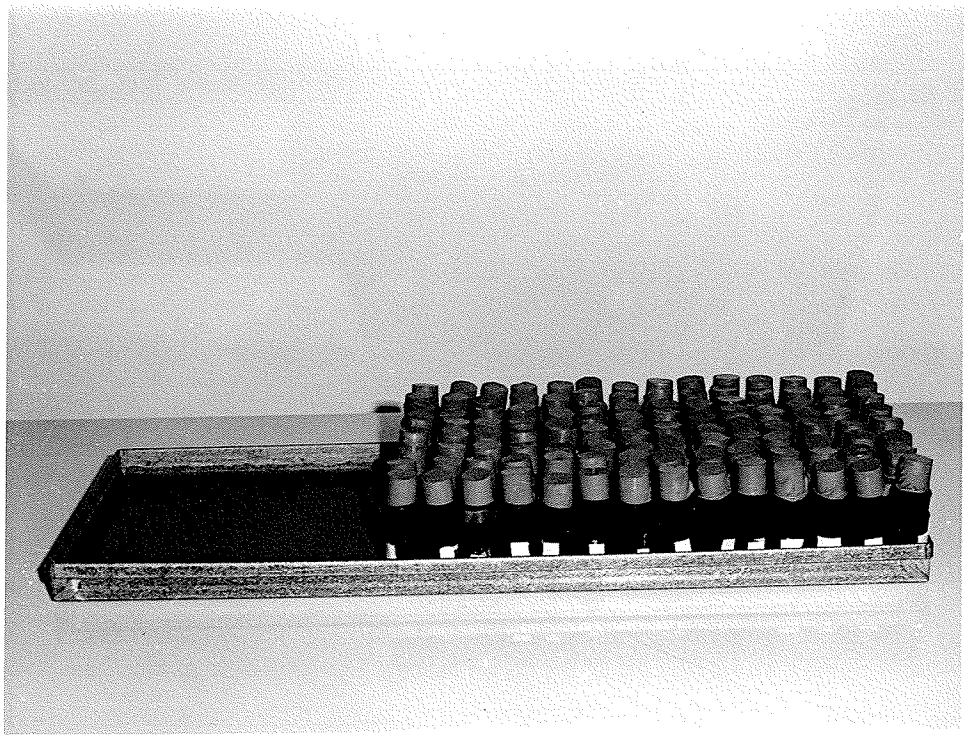

Fig. 4. Feeding cages, containing aphids, set in a tray with water to maintain higher relative humidity.

CHAPTER IV

RESULTS

AZIRIDINES

Aziridines have shown promise as insect chemosterilants. In this study, ten aziridines were investigated for their effects on the mortality and fecundity of the pea aphid.

TEPA

The chemical name of tepa, also sometimes referred to as aphoxide or APO, is tris(1-aziridinyl) phosphine oxide. Tepa is not only an important insect chemosterilant but also has been used in cancer chemotherapy and in the fabric industry. Toxic effects in man and laboratory animals with tepa administered orally or by injection have been recorded at dosages as low as 75 to 0.5 mg./kg. (Anonymous, 1960).

Results of tests with tepa are shown in Tables III, IV, V and VI. Tepa caused a high mortality to feeding nymphs at a dosage of 0.1%, some at 0.05 and 0.025%, but none at 0.01--0.001% (Tables IV, V). Reproduction was inhibited at all dosages from 0.1--0.0025% and only partly at 0.001% (Table III and Figure 13). Ovaries of the pea aphid affected by tepa are shown in Figure 8.

As a result of the feeding test on third instar nymphs with tepa, the question arose as to whether the pea aphid could be sterilized by feeding tepa for a 24 hour period to second instar nymphs. A test was

therefore conducted in which tepa at 0.05% was fed for 24 hours to second instar nymphs (72-84 hours of age). Table VI shows that 11 out of 39 females were completely sterilized while the fecundity in the remaining 28 females was comparatively lower than among the untreated females. It seems that the difference in the amount of uptake of diet during a 24 hour period by nymphs gave varying inhibitions of reproduction. It is, however, evident that feeding for 24 hours by second instar nymphs of the pea aphid on a diet containing tepa at 0.05% can induce sterility effectively.

APHOLATE

Apholate, a hexafunctional aziridine, has the chemical name 2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine. Since apholate has low solubility in water, a particular care was taken that all material dissolved (Chamberlain 1962). Table VII shows that apholate caused no appreciable mortality to the feeding nymphs at any dosages from 0.1--0.001%. Reproduction was inhibited at dosages from 0.1--0.005%, but not at 0.0025 or 0.001%. Results of the test with apholate are also presented in Figure 14. The effect of apholate on the ovaries was similar to that caused by tepa (Figure 9).

METEPA

The chemical name of metepa is tris(2-methyl-1-aziridinyl) phosphine oxide. It is a methyl derivative of tepa and has also been referred to as methaphoxide or MAPO. Metepa caused such high mortality to feeding

nymphs at dosages above 0.01% that no young could be reared to adult stage (Table VIII). The fecundity at 0.01--0.0025% differed significantly from that in the control. The difference in fecundity was presumably due to the toxic effect of metepa. The dissection of ovaries from the treated females did not show any morphological abnormalities.

P, P-BIS(1-AZIRIDINYL)-N-ETHYL-PHOSPHINIC AMIDE (ENT-50,787)

Results of tests with ENT-50,787 are presented in Tables IX and X. It caused complete mortality to feeding nymphs at dosages of 0.1%, 50 per cent at 0.05%, but none at 0.025--0.005%. Due to the deleterious effect of the compound at the 0.05% level, high adult mortality occurred during the reproductive period. Reproduction was completely inhibited at concentrations of 0.05 and 0.025%, and only partly at 0.01 and 0.005%. It is evident from Figure 10 that this compound also inhibited ovarian growth.

P, P-BIS(1-AZIRIDINYL)-N, N-DIMETHYL PHOSPHINIC AMIDE (ENT-50,990)

ENT-50,990 was so insecticidal to the feeding nymphs at a concentration as low as 0.005% that no nymphs survived to adult stage. Lower concentrations were not tested (Table XI).

P-(1-AZIRIDINYL)-N,N,N',N'-TETRAMETHYL PHOSPHONIC DIAMIDE (ENT-50,991)

Data pertaining to the effects of ENT-50,991 on the pea aphid are shown in Table XII. The material at levels of 0.025% and above caused

complete mortality to feeding nymphs. ENT-50,991 did not inhibit reproduction either at 0.01 or 0.005%. However, there was a significant decrease in fecundity among aphids treated at 0.01% compared to that in the control.

P, P-BIS(1-AZIRIDINYL)-N-BUTYL
PHOSPHINIC AMIDE (ENT-51,028)

Results of a test with ENT-51,028 are shown in Table XIII. The compound proved so toxic at dosages as low as 0.025% that no aphids lived to reproduce. Females treated at 0.01 and 0.005% produced much fewer progeny compared to untreated females.

P, P-BIS(1-AZIRIDINYL)-N-PROPYL-
PHOSPHINIC AMIDE (ENT-51,253)

Results obtained by feeding ENT-51,253 to the pea aphid, from early third instar to adult stage, are presented in Table XIV. The compound caused complete mortality to feeding nymphs at concentrations above and including 0.025%. The survival at 0.01% was only 30.0 per cent. Fecundity data show that reproduction was partly inhibited at concentrations of 0.01 and 0.005%.

P, P-BIS(1-AZIRIDINYL)-N-METHYL-
PHOSPHINIC AMIDE (ENT-51,254)

Table XV shows the results obtained by feeding ENT-51,254 to the

pea aphid from early third instar to adult stage. The compound caused complete mortality to feeding nymphs at 0.025%. It was somewhat toxic at 0.01%, while no appreciable mortality occurred to the feeding nymphs at 0.005 or 0.0025%. Reproduction was inhibited at dosages of 0.01 and 0.005% but only partly at 0.0025%. The effect of ENT-51,254 on the ovaries was similar to that caused by apholate and tepa (Figure 11).

P, P-BIS(1-AZIRIDINYL)-N-ISOPROPYL-PHOSPHINIC AMIDE (ENT-51,256)

ENT-51,256 was fed at levels of 0.2--0.005% in a chemical diet to the pea aphid from early third instar to adult stage. Results obtained are shown in Tables XVI and XVII. The data show that the compound was highly toxic to feeding nymphs at concentrations of 0.2--0.05%, while at levels between 0.025--0.005%, it was only slightly toxic. Treated females were weak compared to untreated females. Sterility was induced at dosages of 0.025 and 0.01% while at 0.005%, there was great reduction in fecundity. On dissection, the ovaries of females fed ENT-51,256 were very small and did not contain any developing embryos (Figure 12).

TABLE III

EFFECT OF TEPA FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE[a]

Dosage (% in diet)	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. no. progeny per female produced in 7 days	
				born alive	born dead
0.1	6	0	-	-	-
.05	39	20	-	0.0*	0.05
.025	47	41	-	.0*	.12
.01	56	47	0-2	.09*	.78
.005	50	45	0-6	.64*	1.80
.0025	31	27	0-15	2.55*	2.59
.001	30	27	10-21	15.25*	0.07
control	44	40	16-36	22.15	.10

[a]Average of two tests

*Significantly different to control at 0.01

TABLE IV

EFFECT OF TEPA FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE (FIRST TEST)

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage
0.1	60	6	10.0
.05	60	30	50.0
.025	60	39	65.0
.01	60	47	78.33
.005	60	42	70.0
.0025	60	42	70.0
.001	60	43	71.66
control	60	44	73.33

TABLE V

EFFECT OF TEPA FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE (SECOND TEST)

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage
0.05	50	34	68.0
.025	50	35	70.0
.01	50	38	76.0
.005	50	40	80.0
.0025	50	39	78.0
.001	50	42	84.0
control	95	82	86.1

TABLE VI

EFFECT OF TEPA 0.05% ON THE FECUNDITY OF THE PEA APHID, FED IN A CHEMICAL DIET AT SECOND INSTAR (72-84 HOURS OF AGE) FOR ONE DAY (24 HOURS)

Treatment	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	No. of females completely sterilized	Range of fecundity (live nymphs)	Av. no. progeny per female produced in 7 days	
					born alive	born dead
Tepa 0.05%	40	39	11	0-20	10.85	0.15
Control	41	38	nil	13-22	16.52	0.05

TABLE VII

EFFECTS OF APHOLATE FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. no. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	45	90.0	20	20	-	0.0 *	0.15
.05	50	42	84.0	40	28	-	.0 *	.10
.025	50	42	84.0	40	33	0-3	.15*	.60
.01	50	46	92.0	40	32	0-9	1.43*	1.75
.005	40	35	87.5	20	20	0-6	1.05*	1.40
.0025	50	41	82.0	20	14	11-27	18.0	0.21
.001	50	45	90.0	20	19	10-29	14.63*	.36
control	50	44	88.0	20	15	15-25	19.73	.13

*Significantly different to control at 0.01

TABLE VIII

EFFECTS OF METEPA FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. no. progeny per female produced in 7 days	
							born alive	born dead
0.01	60	17	28.3	15	10	5-14	10.30*	0.10
.005	60	38	63.3	20	20	11-22	16.65*	.0
.0025	60	41	68.3	18	16	11-26	19.37	.06
.001	60	41	68.3	15	15	11-29	22.40	.06
control	60	44	73.3	44	40	16-36	22.15	.10

*Significantly different to control at 0.01

TABLE IX

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-ETHYLPHOSPHINIC AMIDE (ENT-50,787) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.2	100	0	0.0	0	-	-	-	-
.1	100	0	.0	0	-	-	-	-
.05	100	50	50.0	21	5	-	0.0*	0.80
.025	100	94	94.0	27	27	-	.0*	.0
control	100	90	90.0	23	20	13-25	20.75	.0

* Significantly different to control at 0.01

TABLE X

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-ETHYLPHOSPHINIC AMIDE (ENT-50,787) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.01	50	46	92.0	25	23	0-13	6.57*	4.83
0.005	50	48	96.0	25	23	0-20	11.09*	5.35
control	50	48	96.0	25	23	21-36	26.0	0.0

*Significantly different to control at 0.01

TABLE XI

EFFECT OF P,P-BIS(1-AZIRIDINYL)-N,N-DIMETHYLPHOSPHINIC AMIDE (ENT-50,990) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage
0.1	50	0	0.0
.05	50	0	.0
.025	50	0	.0
.01	50	0	.0
.005	50	4	8.0
control	50	45	90.0

TABLE XII

EFFECTS OF P-(1-AZIRIDINYL)-N,N,N',N'-TETRAMETHYLPHOSPHONIC DIAMIDE (ENT-50,991) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.05	50	0	-	-	-	-	-	-
.025	50	0	-	-	-	-	-	-
.01	50	40	80.0	25	21	16-30	23.10*	-
.005	50	44	88.0	25	23	15-37	25.70	-
control	50	48	96.0	25	23	21-36	26.0	-

*Significantly different to control at 0.05

TABLE XIII

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-BUTYLPHOSPHINIC AMIDE (ENT-51,028) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.025	50	5	10.0	5	-	-	-	-
.01	50	37	74.0	25	23	1-18	9.83*	4.65
.005	50	47	94.0	25	21	7-22	15.67*	1.71
control	50	48	96.0	25	23	21-36	26.0	0.0

*Significantly different to control at 0.01

TABLE XIV

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-PROPYLPHOSPHINIC AMIDE (ENT-51,253) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.025	50	0	0.0	-	-	-	-	-
.01	50	15	30.0	15	7	0-7	6.14*	3.29
.005	50	43	86.0	25	24	0-18	6.47*	4.83
control	50	48	96.0	25	23	21-36	26.0	0.0

*Significantly different to control at 0.01

TABLE XV

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-METHYLPHOSPHINIC AMIDE (ENT-51,254) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.025	50	0	-	-	-	-	-	-
.01	50	32	64.0	25	20	-	0.0	0.45
.005	50	40	80.0	25	25	0-3	.16*	1.20
.0025	50	42	84.0	25	18	0-21	11.0 *	1.41
control	50	44	88.0	25	25	14-30	21.96*	0.08

*Significantly different to control at 0.01

TABLE XVI

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-ISOPROPYLPHOSPHINIC AMIDE (ENT-51,256) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.2	100	0	0.0	-	-	-	-	-
.1	100	0	.0	-	-	-	-	-
.05	100	43	43.0	-	-	-	-	-
.025	100	72	72.0	20	15	-	0.0*	0.13
control	100	90	90.0	23	20	13-25	20.75	.0

*Significantly different to control at 0.01

TABLE XVII

EFFECTS OF P,P-BIS(1-AZIRIDINYL)-N-ISOPROPYLPHOSPHINIC AMIDE (ENT-51,256) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.01	50	40	80.0	25	25	-	0.0 *	1.96
.005	50	40	80.0	25	25	0-8	2.36*	2.48
control	50	45	90.0	25	22	18-30	23.77	0.0

* Significantly different to control at 0.01

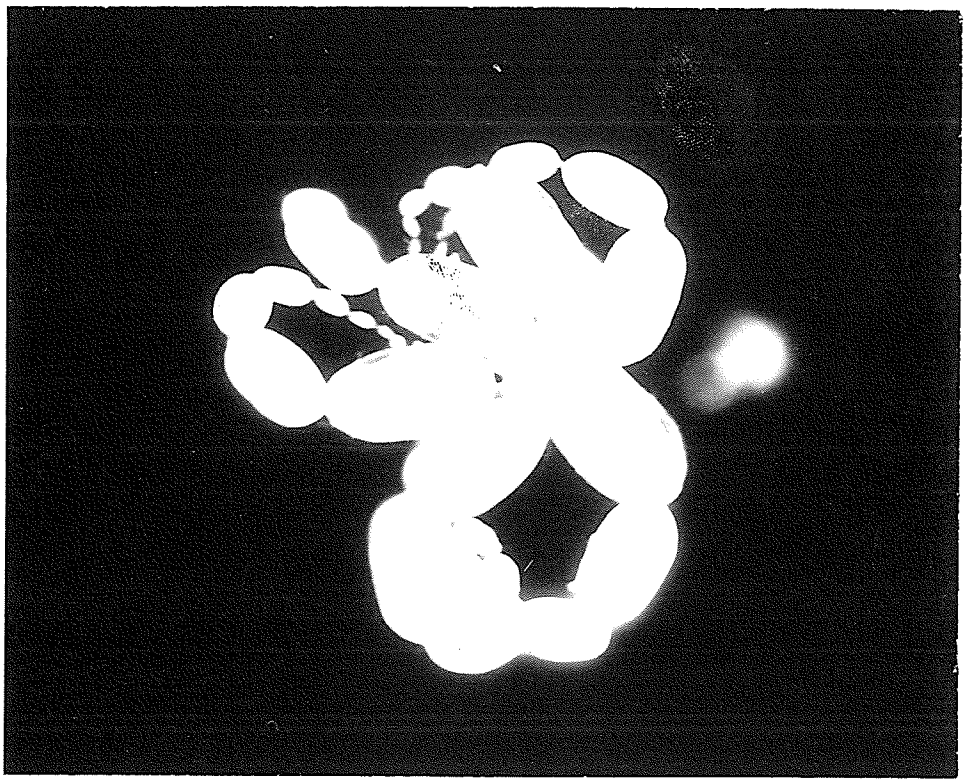

Fig. 5. Normal ovariole development of the pea aphid at early third instar.

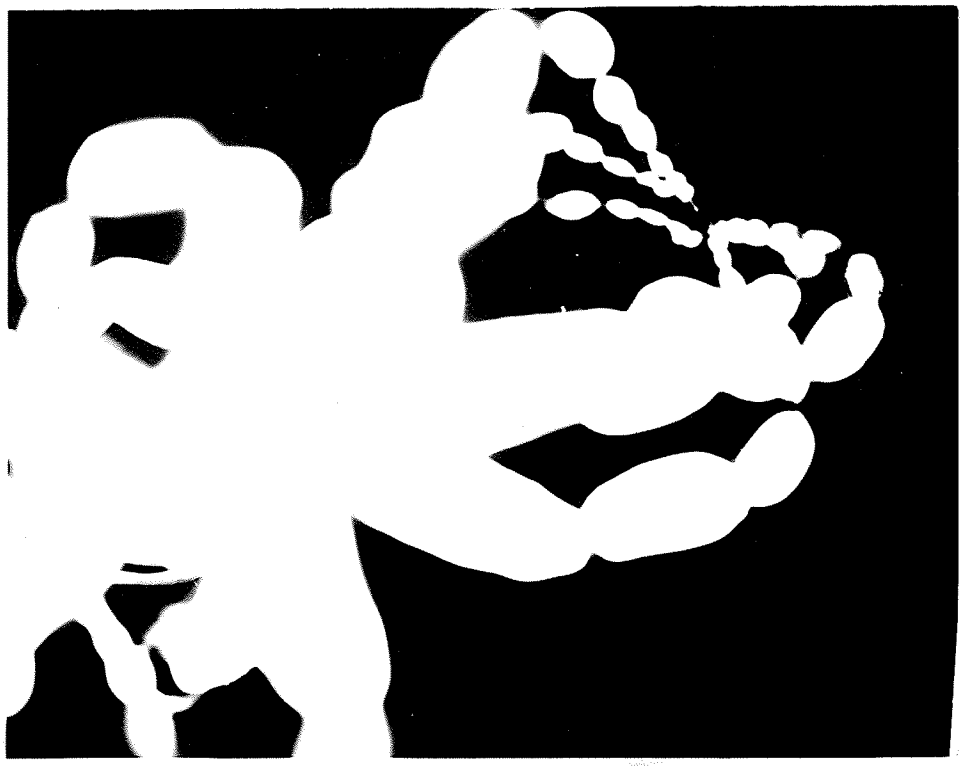

Fig. 6. Ovaries of the adult pea aphid reared on a synthetic diet.

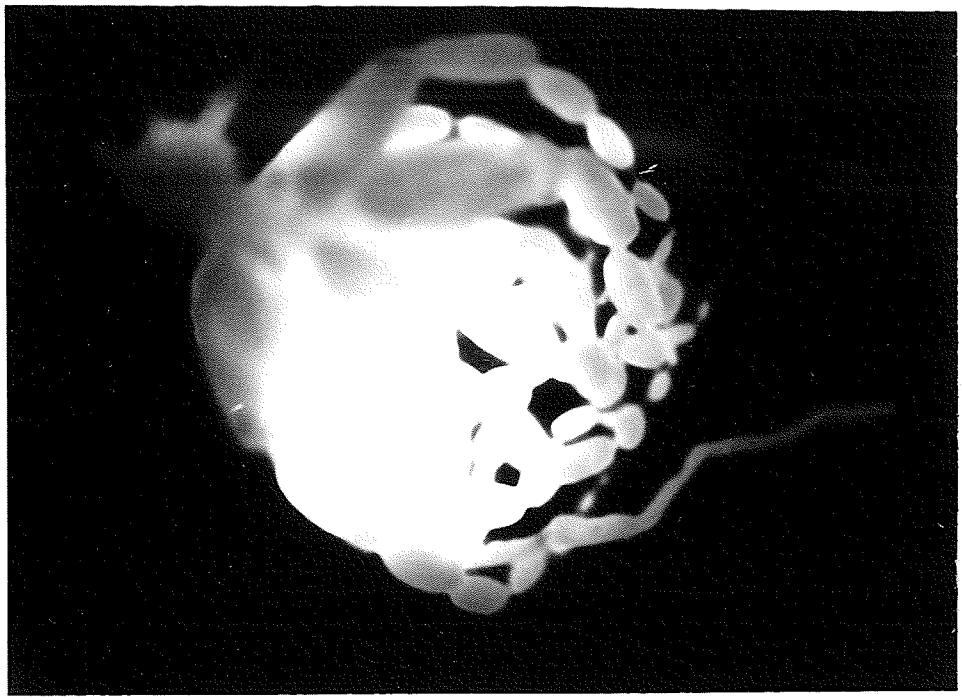

Fig. 7. Ovaries of the adult pea aphid reared on a broad bean plant, Vicia faba L.

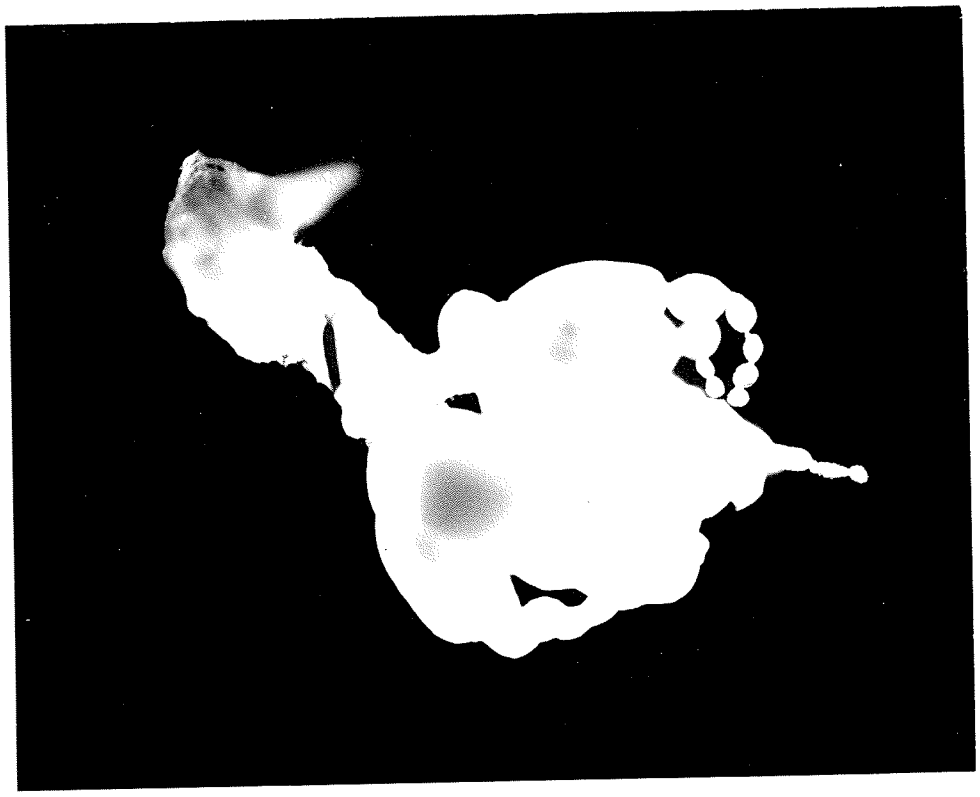

Fig. 8. Ovaries of the adult pea aphid affected by feeding on a synthetic diet containing tepa.

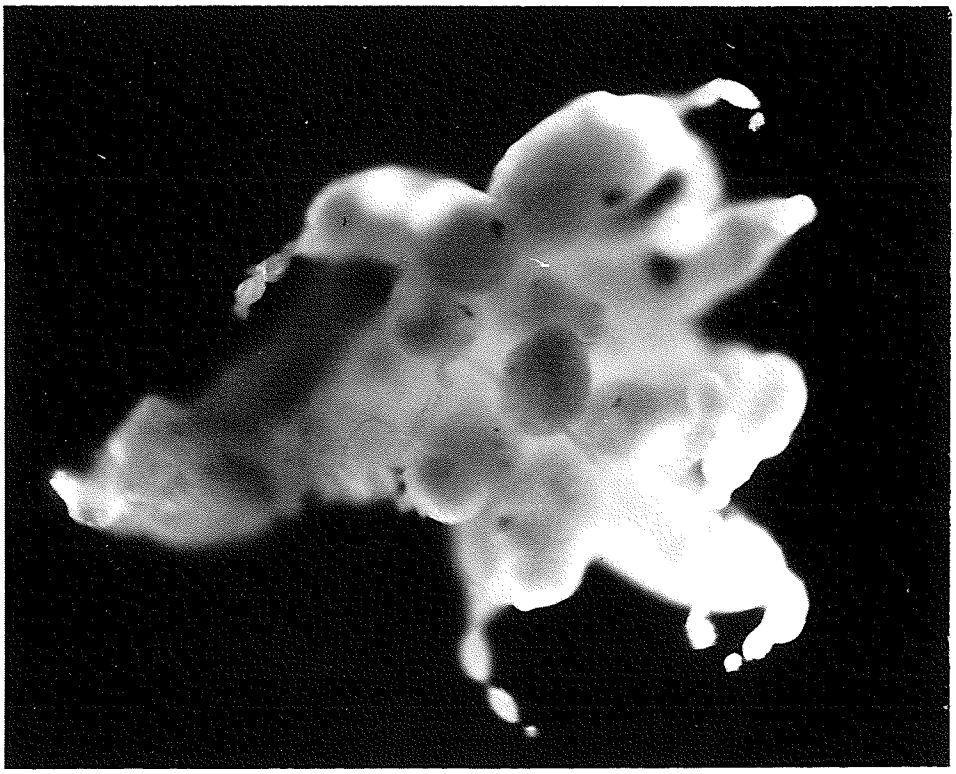

Fig. 9. Ovaries of the adult pea aphid affected by feeding on a synthetic diet containing apholate.

Fig. 10. Ovaries of the adult pea aphid affected by feeding on a synthetic diet containing P,P-bis(1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787).

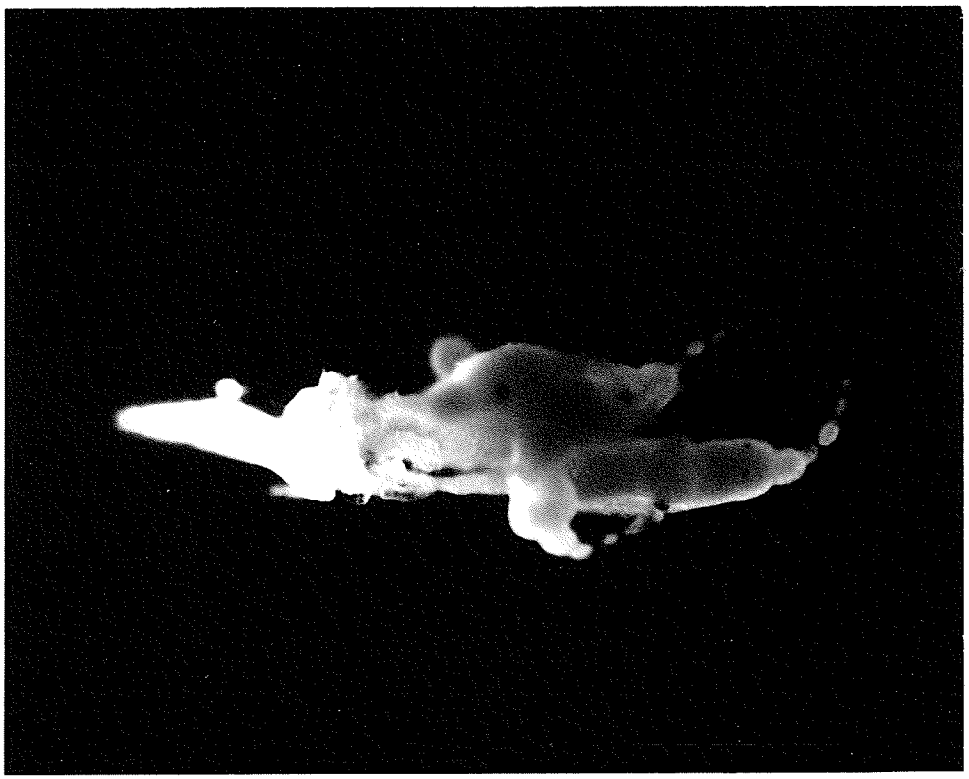

Fig. 11. Part of the ovaries of the adult pea aphid fed on a synthetic diet containing P,P-bis(1-aziridinyl)-N-methylphosphinic amide (ENT-51,254). Figure shows full development of embryos posteriorly and total lack of development anteriorly.

Fig. 12. Ovaries of the adult pea aphid affected by feeding on a synthetic diet containing P,P-bis(1-aziridinyl)-N-isopropylphosphinic amide (ENT-51,256).

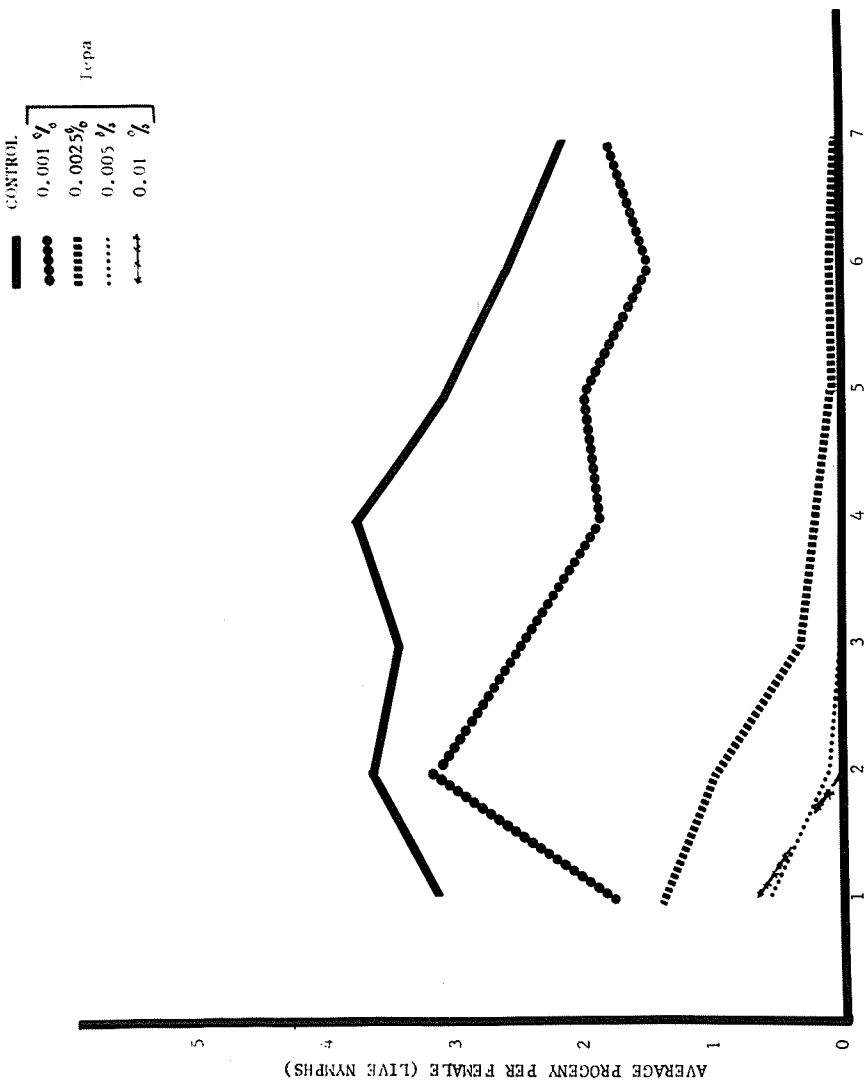

Fig. 13. Effect of tepa on the reproduction of the pea aphid, fed in a chemical diet from early third instar to adult stage.

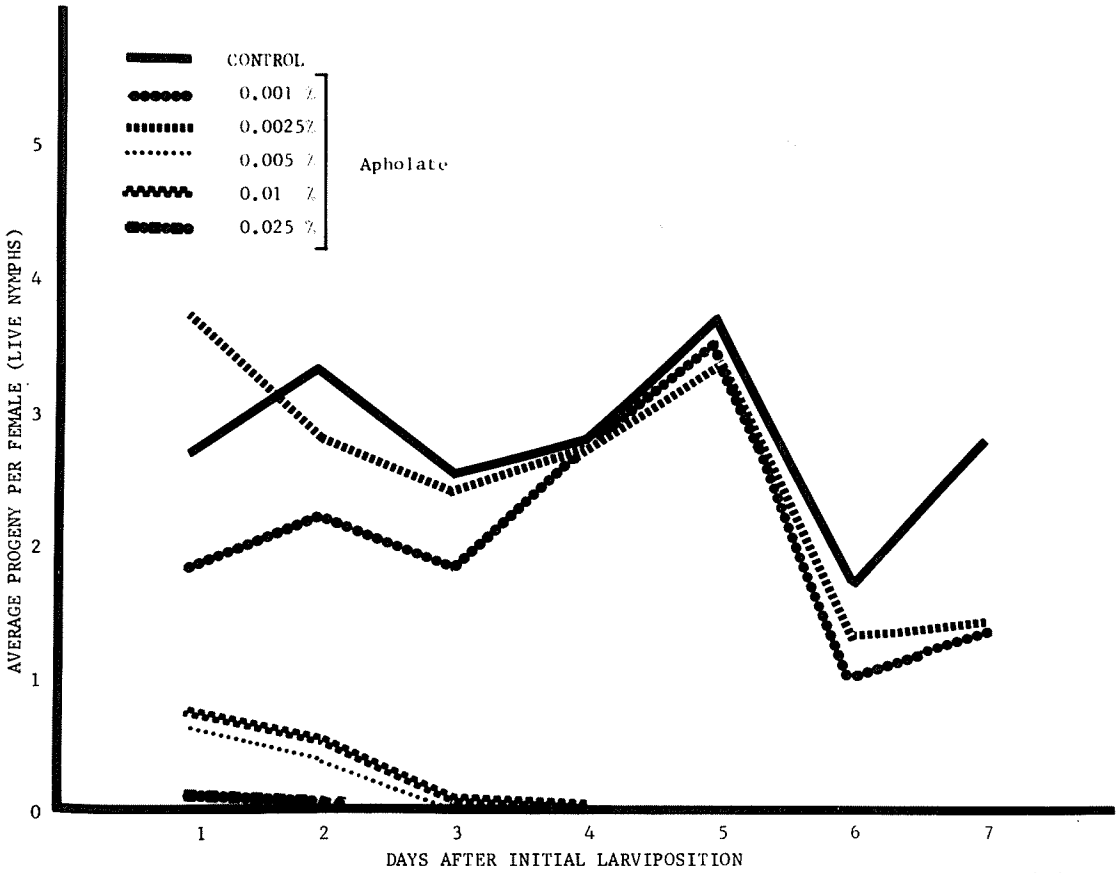

Fig. 14. Effect of apholate on the reproduction of the pea aphid, fed in a chemical diet from early third instar to adult stage.

ANTIMETABOLITES

In the present study, two antimetabolites, methotrexate and 5-fluorouracil were investigated for their effects on the pea aphid.

METHOTREXATE

Methotrexate (=amethopterin), a folic acid antagonist, is chemically known as N-[p-{[(2,4-diamino-6-pteridinyl)methyl] methylamino} benzoyl] glutamic acid. The results of feeding methotrexate are presented in Table XVIII. These results show that methotrexate caused slight mortality to feeding nymphs at dosages of 0.025, 0.05 and 0.1%. At concentrations of 0.2 and 0.4% high mortality of the feeding nymphs occurred. Females which survived these treatments appeared normal. Data on fecundity indicate that methotrexate acted as an effective sterilant at all the dosages tested. At a concentration of 0.025%, 6 among 31 females deposited some live nymphs during the first one or two days of larviposition. Sterile females produced only dead embryos and the sterility induced at these dosages was irreversible.

5-FLUOROURACIL

5-Fluorouracil is one of the pyrimidine antagonists. The effects of feeding 5-fluorouracil for two days to the pea aphid are given in Table XIX. Results show that there was no toxicity at any of the dosages tested. The average number of live progeny produced per female in seven days was

1.73 at 0.05% and 2.77 at 0.025%. Large numbers of dead nymphs were deposited by the treated females. In the second test, 5-fluorouracil was administered to the pea aphid at levels of 0.05 and 0.025% for four days. Data presented in Table XX show that there was complete sterility caused to aphids at 0.05%. At 0.025%, 19 females produced no living young, while in the case of 3 females some nymphs were produced during the first two days of larviposition. One would expect that some harm should have been done to the embryos, that had developed anlagen of appendages, but the compound did not interfere with normal development of these embryos, and they were born alive. Results indicate that 5-fluorouracil was an effective sterilant against the pea aphid.

LaBrecque et al (1960) maintained adult house flies throughout a testing period on diets containing the test chemicals. A few chemicals including 5-fluorouracil and methotrexate were found to be chemosterilants. Methotrexate and 5-fluorouracil have been generally shown as female insect chemosterilants (Crystal 1963; Grosch 1963; Keiser et al 1965; Mitlin and Baroody 1958; Painter and Kilgore 1964). Kilgore and Painter (1966) in their recent study observed that no 5-fluorouracil-2-C^{14} was incorporated into sperm, while a considerable amount was incorporated into the eggs.

Kilgore and Painter (1962) using 5-fluorouracil-2-C^{14} established that there is a correlation between the amount of C^{14} incorporated in the eggs and egg viability. They reported that the largest quantity of label was found in eggs deposited during the first day of oviposition and each following day of the oviposition period the amount of C^{14} found in the eggs decreased considerably until very little was present in the eggs

laid after the fourth day. Consequently sterility was partial and temporary. From the results of another study, Painter and Kilgore (1964) observed that methotrexate and 5-fluorouracil, at 0.01 and 0.05%, respectively, were temporary sterilants when given to the house fly for 48 hours after emergence. 5-Fluoroorotic acid was found to induce permanent sterility, without oviposition, when fed at a level of 1.0% for 48 hours after emergence of house flies (Painter and Kilgore 1965).

TABLE XVIII

EFFECTS OF METHOTREXATE FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.4	50	17	34.0	17	8	-	0.0 *	4.0
.2	50	25	50.0	24	22	-	.0 *	2.0
.1	50	34	68.0	33	33	-	.0 *	3.42
.05	50	38	76.0	35	31	-	.0 *	4.26
.025	50	38	76.0	31	28	0-8	.89*	5.71
control	50	40	80.0	33	25	13-25	20.68	0.08

*Significantly different to control at 0.01

TABLE XIX

EFFECTS OF 5-FLUOROURACIL FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR TWO DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
.05	50	46	92.0	25	22	0-11	1.73*	4.32
.025	50	46	92.0	26	22	0-15	2.77*	6.86
control	50	48	96.0	25	21	15-39	25.90	0.09

*Significantly different to control at 0.01

TABLE XX

EFFECTS OF 5-FLUOROURACIL FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR FOUR DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
.05	50	40	80.0	27	22	-	0.0 *	0.05
.025	50	41	82.0	23	22	0-4	0.32*	2.45
control	50	46	92.0	27	22	19-31	23.64	0.0

*Significantly different to control at 0.01

MISCELLANEOUS COMPOUNDS

TRIAZINES

In the present investigation, seven triazine derivatives were tested for their effects on the pea aphid.

HEMEL

Hemel, hexamethylmelamine or HMM, has a chemical name 2,4,6-tris (dimethylamino)-s-triazine. This compound has a low solubility in water and its LD_{50} for rats is 265 mg./kg. The results of feeding hemel are shown in Table XXI. None of the feeding nymphs survived at concentrations above 0.025%. At rates of 0.025 and 0.01%, the per cent survival of feeding nymphs to adult stage was 78.0 and 84.0, respectively. The adults which survived at 0.025% were extremely weak; out of 20 females, only 4 survived 7 days larviposition period. Fecundity at 0.025 and 0.01% was significantly less compared with the control.

HEXAMETHYLMELAMINE, HYDROCHLORIDE

The results obtained with hexamethylmelamine, hydrochloride, are presented in Table XXII. The mortality to feeding nymphs was slight at dosages of 0.1--0.025%. The fecundity of treated females was significantly less, at the 1% level, compared with untreated females.

2, 4-DIAMINO-6-MORPHOLINO-s-TRIAZINE, HYDROCHLORIDE (ENT-51,143)

2,4-Diamino-6-morpholino-s-triazine, hydrochloride was incorporated into the synthetic diet of the pea aphid at concentrations of 0.1--0.005% and fed from early third instar to adult stage. Results presented in Table XXIII indicate that dosages at and above 0.025% were toxic. No nymphs reached adult stage at 0.1%, while dosages of 0.05 and 0.025% caused high mortality of females during the reproductive period. Fecundity at concentrations of 0.05--0.005% differed from that in the check. Inhibition of reproduction was noticed on a few females at 0.05 and 0.025%.

ENT-51,454 (s-TRIAZINE DERIVATIVE)

ENT-51,454 (s-Triazine derivative) fed at levels of 0.1--0.025% to the pea aphid showed no toxicity to the feeding nymphs. At concentrations of 0.1 and 0.05% high mortalities of females occurred during the larviposition period. Fecundity at 0.1 and 0.05% was reduced significantly (Table XXIV).

3,5-DIAMINO-6-PHENYL-1,2,4-TRIAZINE (ENT-60,279)

Results of tests with 3,5-diamino-6-phenyl-1,2,4-triazine are shown in Table XXV. The data show that the compound caused no appreciable

mortality to the feeding nymphs at any dosages between 0.025--0.001%. ENT-60,279 caused complete mortality to feeding nymphs at 0.1 and 0.05%. Reproduction was inhibited at all dosages from 0.025--0.001%. The effect of ENT-60,279 on the ovaries was similar to that caused by aziridines such as tepa and apholate (Figure 15).

ENT-60,289 (1,2,4-TRIAZINE DERIVATIVE)

ENT-60,289 (1,2,4-triazine derivative) was administered at 0.1% for three days (Table XXVI) and at 0.05% for four days (Table XXVII) to determine its effect on the mortality and fecundity of the pea aphid. The compound was not toxic in any of the treatments. Treated females produced significantly fewer progeny compared to untreated females.

ENT-60,345 (s-TRIAZINE DERIVATIVE)

Results of a test with ENT-60,345 are shown in Table XXVIII. The compound proved so toxic at concentrations as low as 0.01% that no nymphs survived to adult stage.

Among the triazine derivatives tested, 3,5-diamino-6-phenyl-1,2,4-triazine proved most effective as a chemosterilant against the pea aphid. Fye et al (1966) investigated a group of triazines against the house fly. They found that 2,4-diamino-6-(2-furyl)-s-triazine was highly effective at concentrations as low as 0.00025% when incorporated into the fly food but failed to sterilize males when administered either in sugar or fly food at

concentrations as high as 1.0%. Borkovec (1966) reported that although this compound proved an effective house fly chemosterilant, it has no significant effects on mosquitoes, screw-worm flies, Mexican fruit flies, and many other insects.

Hemel has been shown more effective on males than on females. Chang et al (1964) found that hemel induced sterility in males of the house fly. Davis and Eddy (1966) observed that hemel causes high but incomplete sterility of males of the little house fly, Fannia canicularis (L.)

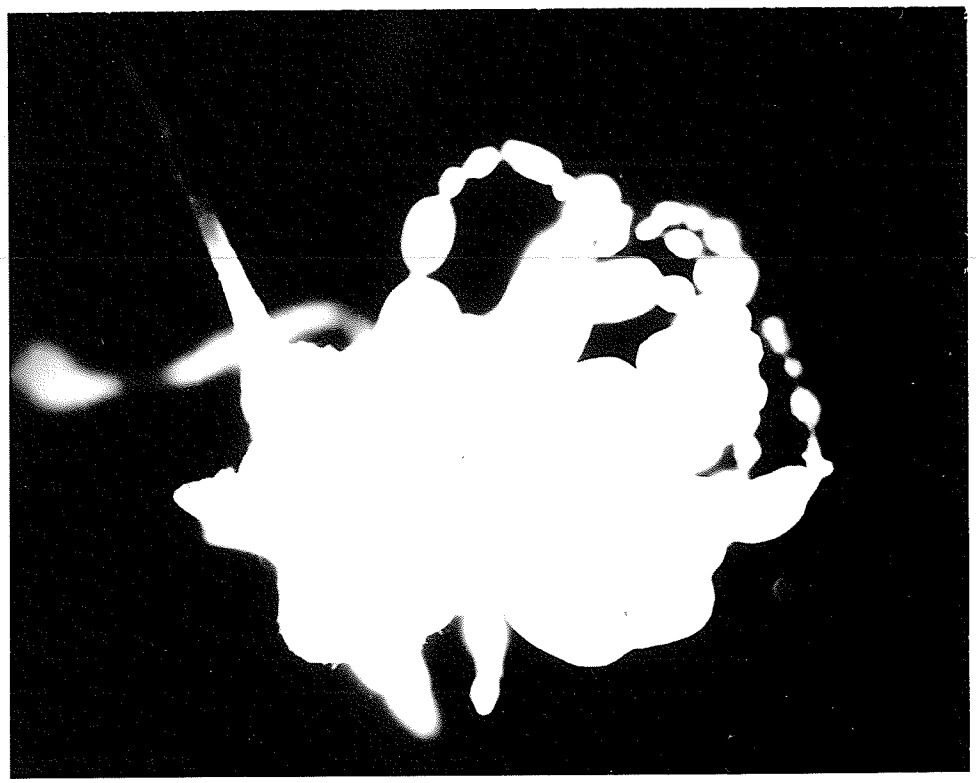

Fig. 15. Ovaries of the adult pea aphid affected by feeding on a synthetic diet containing 3,5-diamino-6-phenyl-1,2,4-triazine (ENT-60,279).

TABLE XXI

EFFECTS OF HEMEL FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR FOUR DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.025	50	39	78.0	20	4	15-21	18.61*	0.38
.01	50	42	84.0	20	17	14-26	20.70*	.00
control	50	47	94.0	20	17	17-30	24.00	.00

*Significantly different to control at 0.05

TABLE XXII

EFFECTS OF HEXAMETHYLMELAMINE, HYDROCHLORIDE (ENT-50,905) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	34	68.0	20	20	7-21	16.80*	0.10
.05	50	37	74.0	20	16	11-19	16.37*	.0
.025	50	37	74.0	20	15	10-23	18.26*	.06
control	50	43	86.0	20	18	16-28	21.00	.0

*Significantly different to control at 0.01

TABLE XXIII

EFFECTS OF 2,4-DIAMINO-6-MORPHOLINO-s-TRIAZINE, HYDROCHLORIDE (ENT-51,143) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR TO ADULT STAGE[a]

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	100	0	0.0	-	-	-	-	-
0.05	100	60	60.0	20	5	0-12	6.60*	1.0
.025	100	75	75.0	49	23	0-19	13.04*	.35
.01	100	88	88.0	60	57	9-25	13.98*	.07
.005	100	88	88.0	40	33	7-23	14.18*	.12
control	100	90	90.0	32	32	12-21	16.59	.03

*Significantly different to control at 0.01

[a]Average of two tests

TABLE XXIV

EFFECTS OF ENT-51,454 (s-TRIAZINE DERIVATIVE) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	49	98	30	12	0-31	16.67*	0.83
.05	50	49	98	30	12	0-31	16.42*	.42
.025	50	49	98	30	20	0-39	22.55	.50
control	50	49	98	48	42	16-31	24-33	.09

*Significantly different to control at 0.01

TABLE XXV

EFFECTS OF 3,5-DIAMINO-6-PHENYL-1,2,4-TRIAZINE (ENT-60,279) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	-	-	-	-	-	-
.05	50	0	-	-	-	-	-	-
.025	50	35	70.0	25	20	-	0.0 *	0.45
.01	50	40	80.0	25	25	-	.0 *	.52
.005	50	42	84.0	25	25	-	.0 *	.84
.0025	50	44	88.0	25	24	0-3	.13*	2.33
.001	50	44	88.0	25	25	0-4	.20*	3.52
control	50	45	90.0	25	22	18-30	23.77	0.0

*Significantly different to control at 0.01

TABLE XXVI

EFFECTS OF ENT-60,289 (1,2,4-TRIAZINE DERIVATIVE) FED IN A CHEMICAL DIET AT 0.1% TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Treatment	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
ENT-60,289	50	50	100.0	30	28	12-26	19.68*	0.0
control	50	50	100.0	26	23	19-31	23.09	.04

*Significantly different to control at 0.05

TABLE XXVII

EFFECTS OF ENT-60,289 (1,2,4-TRIAZINE DERIVATIVE) FED IN A CHEMICAL DIET AT 0.05% TO THE PEA APHID AT EARLY THIRD INSTAR FOR FOUR DAYS

Treatment	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
ENT-60,289	50	49	98.0	27	26	13-28	20.42*	0.04
control	50	50	100.0	26	23	19-31	23.09	.04

*Significantly different to control at 0.01

TABLE XXVIII

EFFECT OF ENT-60,345 (s-TRIAZINE DERIVATIVE) FED IN A CHEMICAL DIET TO THE PEA APHID FROM EARLY THIRD INSTAR TO ADULT STAGE

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage
0.1	50	0	0.0
.05	50	0	.0
.025	50	0	.0
.01	50	0	.0
control	50	46	92.0

DERIVATIVES OF PHOSPHORIC TRIAMIDE

Two derivatives of phosphoric triamide, hempa and thiohempa, were included in tests against the pea aphid.

HEMPA

Hempa, or HMPA (hexamethylphosphoric triamide), one of the derivatives of phosphoric triamide, has drawn considerable attention because it is not an alkylating agent although it has been shown to be an effective insect sterilant. Hempa has a relatively low toxicity for mammals. The acute oral minimum lethal dose for rats is 2640 mg./kg. (Chang et al 1964).

The results of feeding hempa to the pea aphid are given in Table XXIX. At none of the dosages, 01--0.005%, did hempa inhibit reproduction completely. The progeny produced by the treated females were comparatively fewer than in the control.

THIOHEMPA

The chemical name of thiohempa is hexamethylthiophosphoric triamide. This compound was investigated at dosages of 0.1--0.01%, fed in a chemical diet to the pea aphid (Table XXX). Results show that the compound was toxic to the feeding nymphs, and the adults which survived at concentrations of 0.05--0.01% were extremely weak. None among the treated females lived for a seven day reproductive period. However, at a dosage of 0.025%, live and dead nymphs were produced by some adults during the first 3-4 days of reproduction.

Hempa and thiohempa, in these investigations, showed slight activity against the pea aphid. Thiohempa, due to its high toxicity, could not be evaluated properly. Chang et al (1964) demonstrated that hempa was effective as a chemosterilant for male house flies. Similar results were reported by Davis and Eddy (1966) in the little house fly, Glancey (1965) in the boll weevil, and Haynes et al (1966) in the mosquito, Aedes aegypti (L.). Generally the males were shown to be more susceptible to hempa than the females.

TABLE XXIX

EFFECTS OF HEMPA FED IN A CHEMICAL DIET TO THE PEA APHID

FROM EARLY THIRD INSTAR TO ADULT STAGE[a]

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	80	62	77.50	35	29	7-19	12.38*	0.21
.05	80	60	75.0	36	29	8-29	15.70*	.07
.025	80	67	83.75	40	25	13-26	19.76	.05
.01	50	46	92.0	20	15	10-24	17.00*	.20
.005	50	44	88.0	20	20	12-26	17.80*	.05
control	60	56	93.33	40	36	15-28	21.06	.03

[a]Average of two tests

*Significantly different to control at 0.01

TABLE XXX

EFFECTS OF THIOHEMPA FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR FOUR DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	0	-	-	-	-	-
.05	50	2	4.0	4	-	-	-	-
.025	50	6	12.0	12	-	-	-	-
.01	50	33	66.0	20	-	-	-	-
control	50	47	94.0	20	17	17-30	24.0	0.0

TRIPHENYL TIN HYDROXIDE (ENT-28,009)

Triphenyl tin hydroxide has shown favourable results as an insect sterilant (Fye et al 1966; Kissam and Hays 1966; Kenaga 1965). Generally high mortalities were recorded with triphenyl tins. Kenaga (1965) found that triphenyl tins are less effective on males than on females.

Since triphenyl tin hydroxide is not soluble in any of the solvents, a dispersion was prepared with water containing Tween-80 (1 drop of Tween-80 in 10 ml. water). The compound was administered at concentrations of 0.1, 0.05, and 0.01% and the results obtained are presented in Table XXXI. Triphenyl tin hydroxide showed some toxicity at all dosages tested. No nymphs survived to adult stage at 0.1%. The fecundity at 0.05 and 0.01% was reduced considerably.

1-ACETYL-2-THIOUREA (ENT-24,935)

Results of feeding 1-acetyl-2-thiourea to the pea aphid are given in Tables XXXII and XXXIII. The compound was not toxic when fed at 0.05 and 0.025% levels for two days but it resulted in high mortality of the feeding nymphs when administered at these dosages for four days. The fecundity was greatly reduced at all treatments. The compound acted as a temporary sterilant. Data on fecundity represented graphically in Figures 16 and 17, show that large numbers of dead nymphs were born during initial larviposition in treated females, but later live as well as dead nymphs were born. It is evident from the data on fecundity that the chemical

effect was high in the beginning of reproduction but decreased subsequently. It appears likely that the effect of 1-acetyl-2-thiourea was reversed when the treated females began feeding on an untreated diet containing essential nutrients. Presumably 1-acetyl-2-thiourea acts as an antimetabolite in inducing inhibition of reproduction in insects.

Thiourea has been reported to inhibit reproduction in house flies (LaBrecque et al 1960; Konecky and Mitlin 1955; Mitlin and Baroody 1958). Large numbers of thiourea derivatives have been tested against insects. LaBrecque and Gouck (1963) observed that 1,3-dimethyl-2-thiourea was toxic at 2.0%, while at 1.0% it proved toxic as well as sterilant when incorporated into the food of the house fly. Gouck and LaBrecque (1964) and Gouck et al (1963) demonstrated ethylenethiourea as a sterilant affecting only females.

ENT-60,194 (IMIDAZOLE DERIVATIVE)

Table XXXIV shows results obtained by feeding ENT-60,194 to the pea aphid at 0.1, 0.05, and 0.025%. The mortality of feeding nymphs was high at the dosages tested. Only a small number of females survived in different treatments for observations on fecundity. The inhibition of reproduction was caused at dosages of 0.1 and 0.05% while a small number of live nymphs were born at 0.025%. Treated females produced a large number of dead nymphs. Effect of the compound was temporary. Females treated at 0.1 and 0.05% produced dead nymphs during the first seven days of the reproductive period but after this test period treated females, which were

maintained for observation, produced live nymphs. The effect of this compound on the reproduction of the pea aphid was similar to that shown by 1-acetyl-2-thiourea.

2-CHLOROACETAMIDINE HYDROCHLORIDE (ENT-51,325)

Results of feeding 2-chloroacetamidine hydrochloride are shown in Table XXXV. The per cent survival to adult stage at concentrations of 0.1, 0.05, and 0.025% was 64.0, 86.0 and 96.0, respectively, but the females which survived the chemical treatment were weak. High mortality of treated females occurred during larviposition. Fecundity of treated females decreased significantly as compared with that of untreated females.

ENT-60,109 (N-OXIDE OF HETEROCYCLIC NITROGEN COMPOUND)

Results of feeding ENT-60,109 are presented in Table XXXVI. The data show that the compound was so highly toxic at dosages of 0.1 and 0.05% that no nymphs could be reared to adult stage. Mortality at 0.025% was 70.0 per cent. Only a few females survived at this concentration to give fecundity data. Fecundity at 0.025% was reduced considerably and at this treatment, 3 out of 8 females were completely sterilized.

ENT-60,046 (ESTER OF BORIC ACID)

Table XXXVII shows that ENT-60,046 was not insecticidal to the feeding nymphs at dosages between 0.1--0.01%. There was a significant decrease in numbers of live progeny produced by treated females compared with untreated females.

ENT-51,068 [1,1'-(ETHANEDIYLIDENEDINITRO)DI-GUANIDINE, DIHYDROCHLORIDE]

ENT-60,179 (THIADIAZOLE DERIVATIVE)

ENT-60,302 (PYRIMIDINE DERIVATIVE)

ENT-51,068, ENT-60,179, and ENT-60,302 were fed at 0.1% for three days (Table XXXVIII) and at 0.05% for four days (Table XXXIX) to determine their effect on mortality and fecundity of the pea aphid. Results obtained in these tests indicate that none of the three chemicals at mentioned concentrations and feeding durations showed any sterilizing activity or toxicity. However, treated females produced fewer progeny compared to untreated females. Since an average of 1.64 dead nymphs were born by feeding ENT-60,179 at 0.1% for three days, another test was conducted wherein this compound was fed at concentrations of 0.1--0.025%. Results of this test are shown in Table XL. The data show that none of the nymphs feeding at a concentration of 0.1% survived to adult stage.

Females which survived at 0.05% were weak and there occurred high mortality of treated females during larviposition. Fecundity at 0.05 and 0.025% differed significantly from that in the control.

TABLE XXXI

EFFECTS OF TRIPHENYL TIN HYDROXIDE (ENT-28,009) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	0.0	-	-	-	-	-
.05	50	14	28.0	8	6	7-15	11.50*	2.50
.01	50	36	72.0	34	21	0-23	11.67*	0.52
control	50	47	94.0	42	37	15-31	22.05	0.0

*Significantly different to control at 0.01

TABLE XXXII

EFFECTS OF 1-ACETYL-2-THIOUREA (ENT-24,935) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR TWO DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
.05	50	38	76.0	25	18	0-26	13.44*	8.83
.025	50	45	90.0	25	22	0-29	17.05*	5.82
control	50	48	96.0	25	21	15-39	25.90	0.09

*Significantly different to control at 0.01

TABLE XXXIII

EFFECTS OF 1-ACETYL-2-THIOUREA (ENT-24,935) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR FOUR DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	-	-	-	-	-	-
.05	50	8	16.0	7	7	0-9	2.57*	15.42
.025	50	11	22.0	8	8	8-18	13.37*	10.63
control	50	48	92.0	27	22	19-31	23.64	.0

*Significantly different to control at 0.01

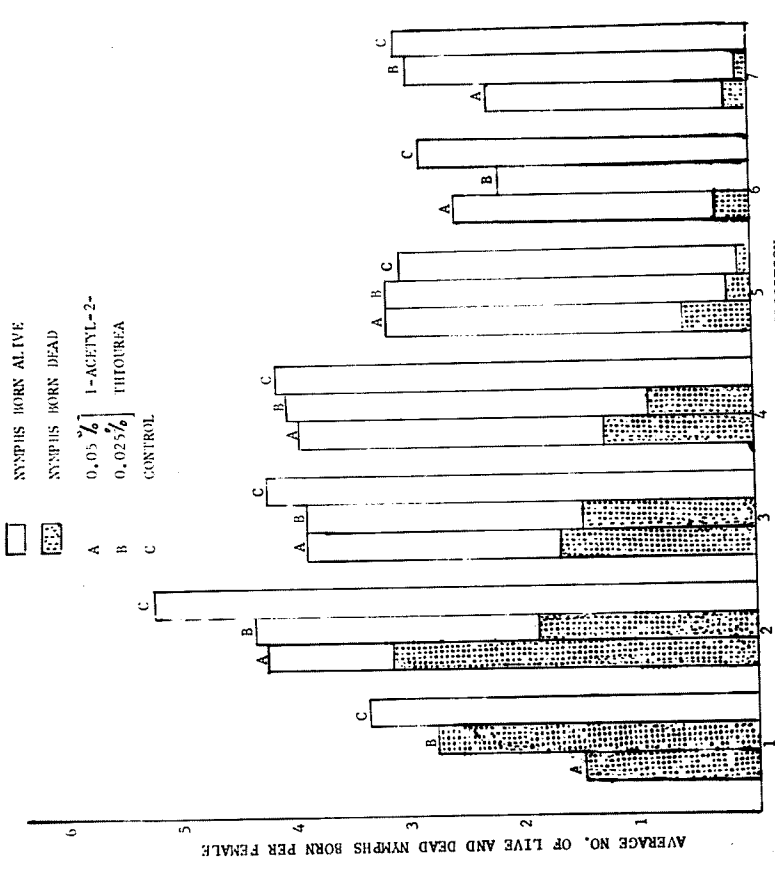

Fig. 16. Effect of 1-acetyl-2-thiourea, on the fecundity of the pea aphid fed for two days

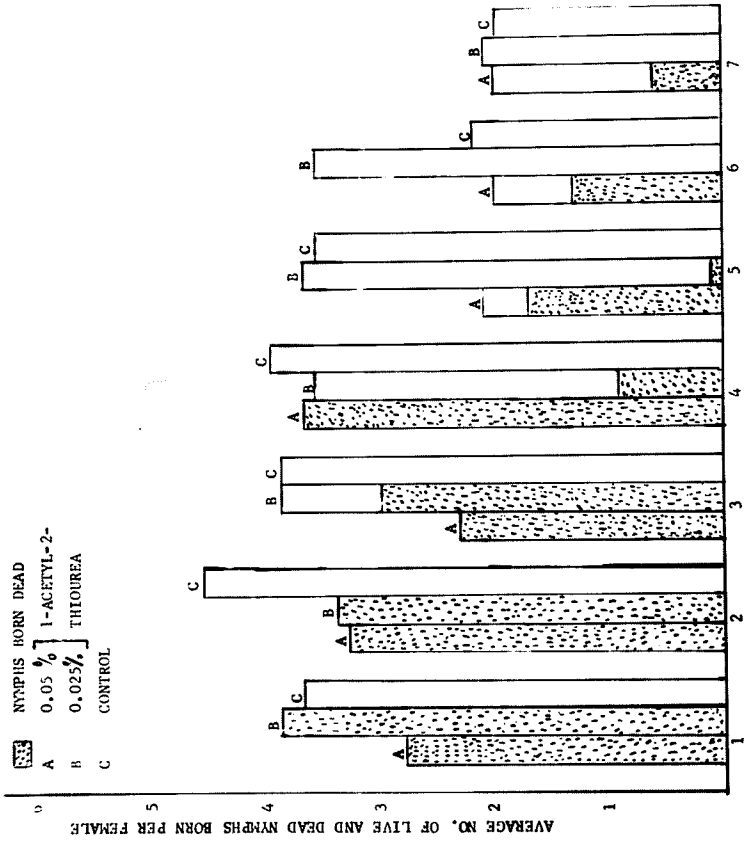

Fig. 17. Effect of 1-acetyl-2-thiourea, on the fecundity of the pea aphid fed for four days

TABLE XXXIV

EFFECTS OF ENT-60,194 (IMIDAZOLE DERIVATIVE) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	2	4.0	2	2	0-1	0.50*	10.0
.05	50	11	22.0	11	8	-	.0 *	13.63
.025	50	11	22.0	11	6	1-7	4.17*	12.50
control	50	49	98.0	48	42	16-31	24.33	0.09

*Significantly different to control at 0.01

TABLE XXXV

EFFECTS OF 2-CHLOROACETAMIDINE HYDROCHLORIDE (ENT-51,325) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	32	64.0	25	nil	-	-	-
.05	50	43	86.0	30	3	1-11	6.0 *	1.33
.025	50	48	96.0	30	18	6-21	13.39*	0.72
control	50	49	98.0	48	42	16-31	24.33	.09

*Significantly different to control at 0.01

TABLE XXXVI

EFFECTS OF ENT-60,109 (N-OXIDE OF A HETEROCYCLIC NITROGEN COMPOUND) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	0.0	-	-	-	-	-
.05	50	0	.0	-	-	-	-	-
.025	50	15	30.0	15	8	0-8	3.25*	2.88
control	50	49	98.0	48	42	16-31	24.33	0.09

*Significantly different to control at 0.01

TABLE XXXVII

EFFECTS OF ENT-60,046 (ESTER OF BORIC ACID) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	44	88.0	39	31	4-25	18.51*	1.41
.05	50	45	90.0	35	31	11-24	18.58*	0.03
.025	50	45	90.0	35	32	9-32	18.00*	.13
.01	50	47	94.0	35	32	11-27	18.94*	.09
control	50	47	94.0	42	37	15-31	22.05	.0

*Significantly different to control at 0.01

TABLE XXXVIII

EFFECTS OF ENT-51,068 [1,1'-(ETHANEDIYLIDENEDINITRO)DI-GUANIDINE, DIHYDROCHLORIDE], ENT-60,179 (THIADAZOLE DERIVATIVE), ENT-60,302 (PYRIMIDINE) FED IN A CHEMICAL DIET AT 0.1% LEVEL TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Chemical	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
ENT-51,068	50	50	100.0	30	27	13-28	18.63*	0.07
ENT-60,179	50	44	88.0	30	25	13-29	19.64**	1.64
ENT-60,302	50	50	100.0	30	28	5-25	17.82*	0.39
control	50	50	100.0	26	23	19-31	23.09	.04

*Significantly different to control at 0.01

**Significantly different to control at 0.05

TABLE XXXIX

EFFECTS OF ENT-51,068 [1,1'-(ETHANEDIYLIDENEDINITRO)DI-GUANIDINE, DIHYDROCHLORIDE], ENT-60,179 (THIADAZOLE DERIVATIVE), ENT-60,302 (PYRIMIDINE) FED IN A CHEMICAL DIET AT 0.05% LEVEL TO THE PEA APHID AT EARLY THIRD INSTAR FOR FOUR DAYS

Chemical	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
ENT-51,068	50	48	96.0	25	23	13-28	20.30	0.0
ENT-60,179	50	46	92.0	26	21	17-43	22.43	.29
ENT-60,302	50	45	90.0	32	28	10-29	20.43	.07
control	50	50	100.0	26	23	19-31	23.09	.04

No significance among treatments

TABLE XL

EFFECTS OF ENT-60,179 (THIADIAZOLE DERIVATIVE) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR SIX DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.1	50	0	0.0	-	-	-	-	-
.05	50	39	78.0	33	10	9-20	10.10*	0.5
.025	50	40	80.0	30	30	11-22	16.47*	.0
control	50	44	88.0	30	29	11-25	18.45	.0

*Significantly different to control at 0.01

TABLE XLI

EFFECT OF CHEMOSTERILANTS FED CONTINUOUSLY, IN A CHEMICAL DIET,

TO THE ADULT PEA APHID DURING LARVIPOSITION PERIOD

Chemosterilant	Dosage (% in diet)	No. of females tested	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
					born alive	born dead
5-Fluorouracil	0.05	21	17	0-7	1.24*	1.41
ENT-51,256	.025	23	1	-	2.0 *	1.0
Methotrexate	.05	22	21	0-14	3.0 *	3.38
ENT-60,279	.025	21	21	1-12	5.67*	5.19
ENT-50,787	.025	23	21	3-19	8.38*	1.90
Apholate	.025	22	21	3-15	9.62*	1.67
Tepa	.025	25	23	5-16	10.52*	0.65
Control	.0	27	20	14-28	20.05	.0

*Significantly different different to control at 0.01

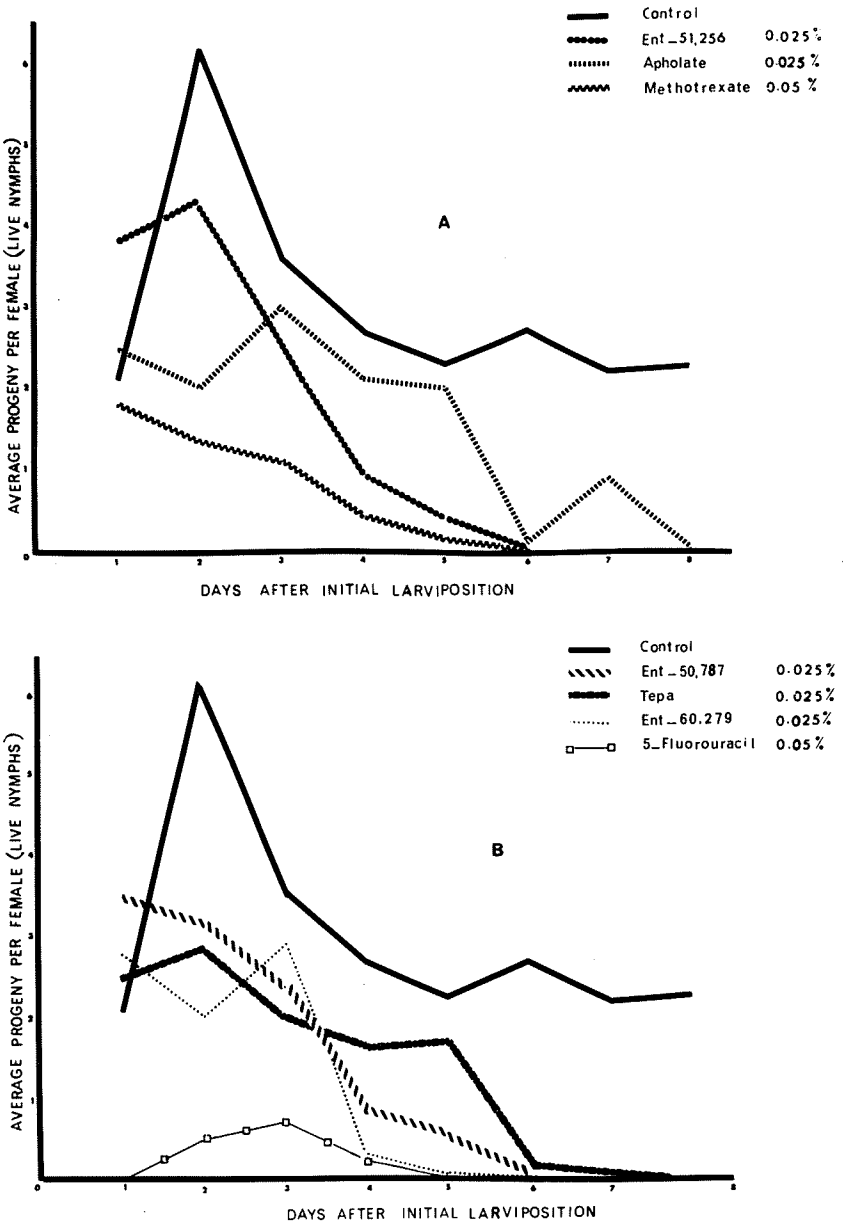

Fig. 18 (A & B). Effect of chemosterilants on the reproduction of the pea aphid fed on a chemical diet during larviposition.

MALEIC HYDRAZIDE

Maleic hydrazide (MH), 1,2-dihydro-3,6-pyridazindione, is used as a plant growth regulator in Agriculture. Zukel (1957, 1963) has made literature summaries on maleic hydrazide for the years 1949-1957 and 1957-1963.

An aqueous solution of MH-30 was used in this test. The results of the test reported in Table XLII show that MH caused high mortality of feeding nymphs at the dosages tested. The surviving females at concentrations of 2.0--0.5% were weak. Fecundity of the treated females differed from that of the untreated females.

Robinson (1959, 1960) observed reduced fecundity of the pea aphid feeding on the broad bean, Vicia faba L., which had absorbed maleic hydrazide through the roots.

Fischnich et al (1958) found a significant reduction in the fertility of rats fed with potato tubers from plants sprayed with MH before harvest compared with rats fed a diet of tubers treated with MH during storage. Yule et al (1966) found that plant metabolites resulting from a MH treatment had no deleterious effect on the reproduction of the house fly and D. melanogaster (Meigen). They also repeated a part of Robinson's (1959, 1960) experiments and confirmed his results. They, however, noted that the growth of MH-treated plants was greatly retarded. They concluded that the reduction in aphid fecundity was indirectly due to nutritional deficiencies in less vigorous MH-treated plants rather than directly due to MH or its metabolites.

CYCOCEL

The chemical name of cycocel, also referred to as chlorocholine chloride, is 2-chloroethyl trimethyl ammonium chloride. This compound is used for causing retardation in plant growth and is used in the United States for producing poinsettias of marketable height and form. Cycocel is acute in oral toxicity to rats (LD_{50} = 1340 mg./kg. for Albino rats).

Cycocel containing 11.8% of active ingredient was used for the test. Data pertaining to the effects of cycocel are presented in Table XLIII. The material caused little or no mortality to the feeding nymphs at the concentrations used. However, appreciable mortality occurred of the treated females during the reproductive period. Fecundity of treated females was significantly lower compared to untreated females.

Emden (1964) reported a reduction in fecundity of the cabbage aphid, Brevicoryne brassicae (L.) on Brussel sprouts treated with cycocel. He suggested that the decreased fecundity and depressed rate of increase of apterous females of the cabbage aphid was due to indirect nutritional effects rather than due to toxic properties of any cycocel taken up by the aphid.

"QUEEN SUBSTANCE"

The term "queen substance" was used by Butler (1954) to designate a hormone-like material, secreted by the queen honey bee, (Apis mellifera

L.), which inhibits ovarian growth in attendant worker bees. Butler et al (1961) identified this substance as 9-oxodec-trans-2-enoic acid. "Queen substance" has been shown to inhibit reproduction in other social insects (Carlisle and Butler 1956; Hrd'y et al 1960). Mitlin and Baroody (1958) found no effect on the ovaries of the house fly, either by feeding or injection of queen bee extract. Nayar (1963) reported that injections at 24-hour intervals of either 9-oxodec-trans-2-enoic acid or its salt inhibited ovarian development in house flies.

In this study synthetic "queen substance" (9-oxodec-trans-2-enoic acid) showed high mortality of feeding nymphs at concentrations of 0.2--0.05% (Table XLIV). The data show that treated females produced significantly fewer progeny compared to untreated females.

TABLE XLII

EFFECTS OF MALEIC HYDRAZIDE (MH-30) FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
2.0	50	11	22.0	9	6	3-17	10.50*	0.67
1.0	60	14	23.33	14	13	0-15	8.92*	0.54
.5	60	18	30.0	15	10	2-15	10.10*	.0
control	60	57	95.0	32	26	13-21	17.58	.0

*Significantly different to control at 0.01

TABLE XLIII

EFFECTS OF CYCOCEL FED IN A CHEMICAL DIET TO THE PEA APHID

AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
2.0	60	47	78.33	37	22	1-15	10.36*	0.0
1.0	60	56	93.33	43	21	8-14	13.57*	.05
.5	60	56	93.33	30	17	4-18	13.06*	.12
control	60	57	95.00	32	26	13-21	17.58	.0

*Significantly different to control at 0.01

TABLE XLIV

EFFECTS OF "QUEEN SUBSTANCE" FED IN A CHEMICAL DIET TO THE PEA APHID AT EARLY THIRD INSTAR FOR THREE DAYS

Dosage (% in diet)	No. of nymphs tested	No. surviving to adult stage	Per cent survival to adult stage	No. of females observed for reproduction	No. of females that produced nymphs for 7 days	Range of fecundity (live nymphs)	Av. No. progeny per female produced in 7 days	
							born alive	born dead
0.2	60	15	25.0	15	11	9-15	12.36*	0.0
.1	60	18	30.0	18	17	6-19	13.29*	.0
.05	60	30	50.0	29	27	8-20	14.94**	.04
control	60	57	95.0	32	26	13-21	17.58	.0

*Significantly different to control at 0.01

**Significantly different to control at 0.05

CHAPTER V

DISCUSSION

The potential uses of chemosterilants were discussed by Lindquist (1961) and Knipling (1962). Chemicals inducing sterility in insect species have been adequately reviewed by Borkovec (1964, 1966) and Smith et al (1964). Results of this study suggested that the pea aphid can be sterilized with tepa, apholate, P, P-bis (1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787), P, P-bis (1-aziridinyl)-N-methylphosphinic amide (ENT-51,254), P, P-bis (1-aziridinyl)-N-isopropyl-phosphinic amide (ENT-51,256), methotrexate, 5-fluorouracil, 3,5-diamino-6-phenyl-1,2,4-triazine (ENT-60,279), 1-acetyl-2-thiourea (ENT-24,935), and an imidazole derivative (ENT-60,194) by introducing them into an artificial diet. The first eight chemicals induced permanent sterility while the last two were only temporary sterilants. Among these chemicals, tepa, apholate, methotrexate and 3,5-diamino-6-phenyl-1,2,4-triazine (ENT-51,256) seem to be the best because of the wide margin of safety between the sterilizing and toxic dosages.

In the present investigation, it became apparent that many chemicals including some known chemosterilants (metepa, hemel and hempa) failed to inhibit reproduction in the pea aphid. Borkovec (1966) has suggested that the activity of chemosterilants depends upon the test insect, sex, developmental stage and the mode of administration. Apparently these factors were related to the effectiveness of chemosterilants on the pea aphid.

Results suggested that there was a positive correlation between the presence of test chemicals in the synthetic diet and the fecundity of the pea aphid. Since the synthetic diet is a mixture of essential nutrients, the reduction in fecundity could either be due to direct effect of test chemicals on the aphid metabolism or the chemical changes that consequently occurred in the synthetic diet due to the addition of test chemicals. It could be possible that some of the test chemicals reacted with the constituents of the synthetic diet in an unpredictable manner, and thus became inactive. In addition, for the same reason, the nutritional efficiency of the diet might also be impaired and this could affect the vigour of the aphids.

Crystal and LaChance (1963) found that aziridinyl compounds inhibit ovarian growth greatly in flies 0-4 hours old but only slightly in those 24 hours old. The age of the test insect is quite an important factor in the susceptibility to chemosterilants. The same is true in the case of aphids. The earlier the instar, the more easily are the embryos affected by the chemosterilants. The development of ovaries in aphids starts taking place from the time of birth of the first instar. Uichanco (1924) observed that aphids at birth contain three eggs in each vitellarium, the one located most anteriorly being just in the process of extrusion, and the rest, in a more or less advanced state of development. The oldest is in the early blastoderm stage. With the pea aphid, the early third instar was more susceptible to chemosterilants than the adult stage. It was evident from these studies that the embryos, con-

tained in the ovarioles, that had formed anlagen of appendages were less affected by chemicals. Such embryos continued to develop and were born either dead or alive.

On dissection, the ovaries of females treated with effective chemosterilants exhibited marked reduction in size compared to those of untreated females. The effect of chemosterilants was more prominent on the anterior than on the posterior of the ovarioles. Inhibited ovaries progressively started degenerating on the anterior of the ovarioles while posteriorly they contained fully developed dead embryos which became brownish. Retardation or complete cessation of ovarian development because of chemical effect has been demonstrated in other insects (Kido and Stafford 1966; Keiser et al 1965; Lindquist et al 1964; Mitlin and Baroody 1958; Mitlin et al 1954, 1957; Morgan and LaBrecque 1964; Painter and Kilgore 1965; Shaw and Riviello 1962).

The research reported in this Thesis has demonstrated for the first time that it is possible, by using an artificial feeding medium enclosed by two artificial membranes, to test the use of chemosterilant compounds against an insect which sucks plant sap. In view of previous successes in the practical use of chemosterilants against other insect pests, of crops and livestock, it is appropriate to suggest that the findings in this Thesis may lead to practical uses of chemosterilants against aphids and other insects which feed on plant sap.

A further significant finding is the demonstration of the action of the several chemosterilants on the embryos within the ovarioles.

Most insects produce eggs, but aphids produce living young. There are implications here for further study on the effects of chemosterilants on embryos of other animals.

CHAPTER VI

SUMMARY

In this study, thirty chemosterilants and three growth regulators were tested for their effects on the mortality and fecundity of the pea aphid, Acyrthosiphon pisum (Harris), an economic pest of leguminous crops.

A pea aphid culture was maintained on broad bean plants, Vicia faba L. Except in two experiments, pea aphids at early third instar were used. Tests were conducted in a plant-growth cabinet which maintained a temperature of 69°F., relative humidity of 70±2 per cent and a photoperiod of 16 hours of light and eight hours of darkness.

The feeding cage and chemically defined diet were similar to those of Auclair and Cartier (1963). The test chemicals were incorporated into the synthetic diet and fed to the pea aphid. For each test, the mortality counts were recorded after nymphs had become adults. Adult females were caged individually for larviposition. The cages were examined daily in order to count live and dead larvae produced. The count of progeny was made for seven days from the day of initial larviposition. Data on live progeny of treated and untreated females were subjected to statistical analysis.

Ten aziridines, at different concentrations, were administered from third instar to adult stage. Among the aziridines tested, tepa, apholate, P, P-bis (1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787),

P, P-bis(1-aziridinyl)-N-methylphosphinic amide (ENT-51,254), and P, P-bis(1-aziridinyl)-N-isoprophylphosphinic amide (ENT-51,256) were found effective in inducing sterility in the pea aphid. The sterility caused was permanent.

Tepa was fed at levels of 0.1--0.001%. Tepa inhibited reproduction at dosages of 0.1--0.0025% but caused some mortality to feeding nymphs at dosages higher than 0.025%. Tepa fed at 0.05%, to second instar nymphs (72-84 hours old) for a 24 hour period, caused sterility in 11 out of 39 females. Inconsistency in the induction of sterility was possibly due to difference in the amount of uptake of treated diet to nymphs. Apholate inhibited reproduction at dosages from 0.1--0.005% but caused no mortality to feeding nymphs at any dosages from 0.1--0.001%. P, P-bis(1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787), P, P-bis(1-aziridinyl)-N-methylphosphinic amide (ENT-51,254) and P, P-bis(1-aziridinyl)-N-isopropylphosphinic amide (ENT-51,256) were incorporated into the synthetic diet over a wide range of concentrations. ENT-50,787 was lethal at 0.1% and a delayed toxic effect was observed at 0.05%. ENT-51,254 and ENT-51,256 were lethal at dosages above 0.01%. Reproduction was inhibited with ENT-50,787, ENT-51,254 and ENT-51,256 at dosages of 0.05--0.025%, 0.01--0.005%, and 0.025--0.01%, respectively.

Metepa, P-bis(1-aziridinyl)-N,N,N',N'-tetramethylphosphonic amide (ENT-50,991), and P, P-bis(1-aziridinyl)-N-butylphosphinic amide (ENT-51,028), P, P-bis(1-aziridinyl)-N-propylphosphinic amide

(ENT-51,253) were insecticidal at concentrations above 0.01%. Significant reduction in fecundity was caused by these materials at lower dosages but none caused complete sterility. P, P-bis(1-aziridinyl)-N, N-dimethylphosphinic amide (ENT-50,990) was highly toxic to feeding nymphs at dosages of 0.1--0.005%. No fecundity data could be recorded.

Methotrexate was administered at dosages from 0.4--0.025% for three days. Inhibition of reproduction was caused at all dosages tested but appreciable mortality was observed at dosages of 0.4 and 0.2%. 5-Fluorouracil fed at levels of 0.05 and 0.025% for two or four days induced sterility. Methotrexate and 5-fluorouracil were permanent sterilants against the pea aphid.

Seven triazines were investigated for their sterilant and toxic effects. 3,5-Diamino-6-phenyl-1,2,4-triazine was the only triazine that induced sterility effectively at dosages of 0.025--0.001%. The compound showed some toxicity at 0.025% but was lethal above this dosage. The sterility induced was permanent. Hemel, hexamethylmelamine, hydrochloride (ENT-50,905), 2,4-diamino-6-morpholino-s-triazine, hydrochloride (ENT-51,143), ENT-51,454 (s-triazine derivative), and ENT-60, 289 (1,2,4-triazine derivative) were found to reduce fecundity significantly but none caused sterility. ENT-60,345 (s-triazine derivative) was so toxic at dosages of 0.1--0.01% that no nymphs could be reared to adult stage.

Hempa fed at concentrations of 0.1--0.005% resulted in a significant decrease in fecundity. Some toxic effects were observed at levels of 0.1 and 0.05%. Thiohempa showed a high degree of toxicity to

feeding nymphs at concentrations of 0.1--0.01%. Females which survived the chemical treatment were weak. None of the treated females survived the seven day reproductive period.

Triphenyl tin hydroxide (ENT-28,009), 2-chloroacetamidine hydrochloride (ENT-51,325), ENT-60,109 (N-oxide of a heterocyclic nitrogen compound), ENT-60,046 (Ester of boric acid), 1, 1'-ethanediylidenedinitro)di-, Guanidine, dihydrochloride (ENT-51,068), ENT-60,179 (Thiadozole derivative), and ENT-60,302 (Pyrimidine derivative) reduced fecundity significantly compared with the check. In no instance was sterility induced.

1-Acety-2-thiourea (ENT-24,935) and ENT-60,194 (Imidazole derivative) induced temporary sterility. The effect of these chemicals was reversed when the treated females began feeding on an untreated diet containing essential nutrients. Treated females initially produced dead nymphs but subsequently live as well as dead nymphs were born. Presumably these compounds acted as antimetabolites.

Maleic hydrazide (MH-30) and cycocel were administered at concentrations of 2.0--0.5%. Both materials caused significant reduction in fecundity compared to the control. Maleic hydrazide exhibited considerable toxicity to feeding nymphs and the surviving adults were weak. No noticeable toxicity was observed with cycocel.

Synthetic "queen substance", which was administered at levels of 0.2--0.05%, caused high mortality to feeding nymphs. The fecundity of treated females was reduced significantly compared to untreated females.

A test was conducted, where 5-fluorouracil and methotrexate at 0.05% level, and P, P-bis(1-aziridinyl)-N-ethylphosphinic amide (ENT-50,787), P, P-bis(1-aziridinyl)-N-isopropylphosphinic amide (ENT-51, 256), 3,5-diamino-6-phenyl-1,2,4-triazine (ENT-60,279), apholate and tepa at 0.025% were incorporated into the synthetic diet and fed continuously to adult females to determine the extent of inhibition induced. ENT-51,256 was lethal to feeding females. The reproduction was inhibited by the remaining materials administered but all embryos which had fully developed appendages at the time of administration of chemicals to adults were born either alive or dead.

Adult females treated with effective chemosterilants were dissected in saline water. Ovaries were found atrophied due to the effect of chemosterilants. The effect of chemicals was more pronounced on the anterior of ovarioles. Ovaries inhibited due to chemical effect, progressively showed degeneration. Sterilized females assumed a distinctive translucency of body content.

BIBLIOGRAPHY

Alexander, P. 1960. Radiation-imitating chemicals. Sci. Amer. 202: 99-108.

Anonymous. 1960. Cancer Chemotherapy Reports (8) July, 1960. pp. 156-175.

Auclair, J. L. 1965. Feeding and nutrition of the pea aphid, Acyrthosiphon pisum (Homoptera: Aphidae), on chemically defined diets of various pH. and nutrient levels. Ann. Entomol. Soc. Am. 58: 855-875.

Auclair, J. L. and J. J. Cartier. 1963. Pea aphid: rearing on a chemically defined diet. Science 142: 1068-1069.

Bartlett, M. S. 1936. Square root transformation in analysis of variance. Suppl. J. Roy. Statist. Soc. 3: 68-78.

Baumhover, A. H., A. J. Graham, B. A. Bitter, D. E. Hopkins, W. D. New, F. H. Dudley, and R. C. Bushland. 1955. Screw-worm control through release of sterilized flies. J. Econ. Entomol. 48: 462-466.

Benschoter, C. A. 1966. Reserpine as a sterilant for the Mexican fruit fly. J. Econ. Entomol. 59: 333-334.

Bhalla, O. P. and A. G. Robinson. 1966. Effect of three chemosterilants on the pea aphid fed on an artificial diet. J. Econ. Entomol. 59: 378-379.

Borkovec, A. B. 1964. Insect chemosterilants. Their chemistry and application. Residue Rev. 6: 87-103.

Borkovec, A. B. 1966. Insect chemosterilants. John Wiley and Sons, Inc., New York. pp. 1-143.

Borkovec, A. B., S. C. Chang, and A. M. Limburg. 1964. Effect of pH on sterilizing activity of tepa and metepa in male house flies. J. Econ. Entomol. 57: 815-817.

Borkovec, A. B. and C. W. Woods. 1963. Aziridine chemosterilants. Sulphur-containing aziridines. Adv. Chem. Ser. 41: 47-55.

Bushland, R. C. and D. E. Hopkins. 1953. Sterilization of screw-worm flies with X-rays and gamma rays. J. Econ. Entomol. 46: 648-656.

Butler, C. G. 1954. The method and importance of the recognition by a colony of honeybees (A. mellifera) of the presence of its queen. Trans. Roy. Ent. Soc. (London). 105: 11-29.

Butler, C. G., R. K. Callow, and N. C. Johnston. 1961. The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic-acid, a honeybee pheromone. Proc. Roy. Soc. (London). B 155: 417-432.

Cantwell, G. E. and T. J. Henneberry. 1963. The effect of gamma radiation and apholate on the reproductive tissues of Drosophila melanogaster Meigen. J. Insect Pathol. 5: 251-264.

Carlisle, D. B. and C. G. Butler. 1956. The "queen substance" of honeybee and the ovary inhibiting hormone of crustaceans. Nature 177: 276-277.

Chamberlain, W. F. 1962. Chemical sterilization of the screw-worm. J. Econ. Entomol. 55: 240-248.

Chamberlain, W. F. and C. C. Barrett. 1964. A comparison of the amounts of metepa required to sterilize the screw-worm fly and stable fly. J. Econ. Entomol. 57: 267-269.

Chamberlain, W. F. and E. W. Hamilton. 1964. Absorption, excretion, and metabolism of P^{32}-labeled metepa by screw-worm and stable flies. J. Econ. Entomol. 57: 800-803.

Chamberlain, W. F. and D. E. Hopkins. 1960. Effect of colchicine on screw-worm. J. Econ. Entomol. 53: 1133-1134.

Chang, S. C. 1965a. Chemosterilization and mating behaviour of male house flies. J. Econ. Entomol. 58: 669-672.

Chang, S. C. 1965b. Improved bioassay method for evaluating the potency of chemosterilants against house flies. J. Econ. Entomol. 58: 796.

Chang, S. C. and A. B. Borkovec. 1964. Quantitative effects of tepa, metepa, and apholate on sterilization of male house flies. J. Econ. Entomol. 57: 488-490.

Chang, S. C. and C. W. Kearns. 1962. Effect of sesamex on toxicities of individual pyrethrins. J. Econ. Entomol. 55: 919-922.

Chang, S. C., P. H. Terry, and A. B. Borkovec. 1964. Insect chemosterilants with low toxicity for mammals. Science. 144: 57-58.

Collier, C. W. and J. E. Downey. 1965. Laboratory evaluation of certain chemosterilants against the gypsy moth. J. Econ. Entomol. 58: 649-651.

Crystal, M. M. 1963. The induction of sexual sterility in the screw-worm fly by antimetabolite and alkylating agents. J. Econ. Entomol. 56: 468-473.

Crystal, M. M. 1964a. Insect fertility: inhibition by folic acid derivatives. Science 144: 308-309.

Crystal, M. M. 1964b. Sexual sterilization of screw-worm flies by biological alkylating agents, tretamine and thiotepa. Exp. Parasitol. 15: 249-259.

Crystal, M. M. 1964c. Chemosterilant efficiency of bis(1-aziridinyl) phosphinyl carbamates in screw-worm flies. J. Econ. Entomol. 57: 726-731.

Crystal, M. M. 1965. Sexual sterilization of insects by aerosol administration of alkylating agents. J. Econ. Entomol. 58: 678-680.

Crystal, M. M. 1966. Some structure-activity relationships among aziridinyl antifertility agents in screw-worm flies. J. Econ. Entomol. 59: 577-580.

Crystal, M. M. and L. E. LaChance. 1963. Sexual sterilization of screw-worm flies by the biological alkylating agents. I. Inhibition of ovarian growth and egg production and hatchability. Biol. Bull. 125: 270-279.

Dame, D. A. and H. R. Ford. 1964. Chemosterilization and its permanency in mosquitoes. Nature 201: 733-734.

Dame, D. A. and C. H. Schmidt. 1964. Uptake of metepa and its effects on two species of mosquitoes (Anopheles quadrimaculatus, Aedes aegypti) and house flies (Musca domestica). J. Econ. Entomol. 57: 77-81.

Davich, T. B., J. C. Keller, E. B. Mitchell, P. Huddleston, R. Hill, D. A. Lindquist, G. McKibben and W. H. Cross. 1965. Preliminary field experiments with sterile males for eradication of the boll weevil. J. Econ. Entomol. 58: 127-131.

Davis, H. G. and G. W. Eddy. 1966. Some effects of chemosterilants on the little house fly. J. Econ. Entomol. 59: 993-996.

Edel'man, N. M. and A. M. Efros. 1962. The effect of plant growth-promoting substances on phytophagous insects. Doklady Akademii Nauk SSSR. 142: 1172-1175.

Emden, H. F. van. 1964. Effect of (2-chloroethyl) trimethyl-ammonium chloride on the rate of increase of the cabbage aphid, Brevicoryne brassicae L. Nature 201: 946-948.

Fischnich, O., C. Patzold, and C. Schillar. 1958. The use of growth-regulating substances on potato crops. Eur. Potato J. 1: 25-30.

Fye, R. L., H. K. Gouck, and G. C. LaBrecque. 1965. Compounds causing sterility in adult house flies. J. Econ. Entomol. 58: 446-448.

Fye, R. L., G. C. LaBrecque, and H. K. Gouck. 1966. Screening tests of chemicals for sterilization of adult house flies. J. Econ. Entomol. 59: 485-487.

Geering, Q. A., P. J. Brooker, and J. H. Parsons. 1965. The chemosterilant activity of some substituted phenyl esters of aziridine-1-carboxylic acid. J. Econ. Entomol. 58: 574-575.

Glancey, B. M. 1965. Hempa as a chemosterilant for the yellow mosquito, Aedes aegypti (L.) (Diptera: Culicidae). Mosquito News. 25: 392-396.

Goldsmith, E. D. 1958. The effect of 5-fluoroorotic acid on the development of the fruit fly, Drosophila melanogaster. Anat. Rec. 131: 559.

Goldsmith, E. D. and I. Frank. 1952. Sterility in the female fruit fly, Drosophila melanogaster, produced by the feeding of a folic acid antagonist. Am. J. Physiol. 171: 726-727.

Gouck, H. K. 1964. Chemosterilization of house flies by treatment in the pupal stage. J. Econ. Entomol. 57: 239-241.

Gouck, H. K., M. M. Crystal, A. B. Borkovec, and D. W. Meifert. 1963a. A comparison of techniques for screening chemosterilants of house flies and screw-worm flies. J. Econ. Entomol. 56: 506-509.

Gouck, H. K. and G. C. LaBrecque. 1964. Chemicals affecting fertility in adult house flies. J. Econ. Entomol. 57: 663-664.

Gouck, H. K., D. W. Meifert, and J. B. Gahan. 1963b. A field experiment with apholate as a chemosterilant for the control of house flies. J. Econ. Entomol. 56: 445-446.

Grosch, D. S. 1959. The effects of feeding antimitotic substances to adult female Habrobracon (Microbracon)hebator (Say); (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 52: 294-298.

Grosch, D. S. 1963. Insect fecundity and fertility. Chemically induced decrease. Science 141: 732-733.

Hair, J. A. and T. R. Adkins, Jr. 1964. Sterilization of the face fly, Musca autumnalis with apholate and tepa. J. Econ. Entomol. 57: 586-589.

Hair, J. A. and E. C. Turner, Jr. 1966. Susceptibility of mature and newly emerged face flies to chemosterilization with apholate. J. Econ. Entomol. 59: 452-454.

Harries, F. H. 1961. Effect of certain antibiotics and other compounds on the two-spotted spider mite. J. Econ. Entomol. 54: 122-124.

Harries, F. H. 1963. Effects of some antibiotics and other compounds on fertility and mortality of orchard mites. J. Econ. Entomol. 56: 438-441.

Harries, F. H. and V. J. Mattson. 1963. Effects of some antibiotics on three aphid species. J. Econ. Entomol. 56: 412-414.

Harris, R. L. 1962. Chemical induction of sterility in the stable fly. J. Econ. Entomol. 55: 882-885.

Harris, R. L. and E. D. Frazar. 1966. Chemosterilization of adult horn flies. J. Econ. Entomol. 59: 1171-1173.

Hathaway, D. O., L. V. Lydin, and B. A. Butt. 1966. Effects of tepa on reproduction of codling moths. J. Econ. Entomol. 59: 851-853.

Haynes, J. W., P. A. Hedin, and T. B. Davich. 1966. Hempa and apholate as chemosterilants for the boll weevil. J. Econ. Entomol. 59: 1014-1015.

Hays, S. B. and J. H. Cochran. 1964. Evaluation of compounds affecting the reproductive potential of the plum curculio. J. Econ. Entomol. 57: 217-219.

Hedin, P. A., C. P. Cody, and A. C. Thompson, Jr. 1964. Antifertility effect of the chemosterilant apholate on the male boll weevil. J. Econ. Entomol. 57: 270-272.

Henneberry, T. J., H. H. Shorey, and A. N. Kishaba. 1966. Mating frequency and copulatory aberrations, response to female sex pheromone, and longevity of male cabbage loopers treated with tepa. J. Econ. Entomol. 59: 573-576.

Henneberry, T. J., F. F. Smith, and W. L. McGovern. 1964. Some effects of gamma radiation and a chemosterilant on the Mexican bean beetle. J. Econ. Entomol. 57: 813-815.

Howland, A. F., P. Vail, and T. J. Henneberry. 1965. Effect of chemosterilants on fertility of cabbage loopers. J. Econ. Entomol. 58: 635-637.

Howland, A. F., P. Vail, and T. J. Henneberry. 1966. Results of cage experiments with sterile male releases and a chemosterilant technique for control of cabbage looper populations. J. Econ. Entomol. 59: 194-196.

Hrd'y, I., V. J. A. Novak, and D. S. Krobal. 1960. Influence of the queen inhibitory substance of honeybee on the development of supplementary reproductives in the termite, Kalotermes flavicelis. pp. 172-174 In The Ontogeny of Insects. Acta Symposii de evolutione insectorum, Prague, 1959. Academic Press, New York.

Keiser, I., L. F. Steiner, and H. Kamaski. 1965. Effect of chemosterilants against the Oriental fruit fly, melon fruit fly, and Mediterranean fruit fly. J. Econ. Entomol. 58: 682-685.

Kenaga, E. E. 1965. Triphenyl tin compounds as insect reproduction inhibitors. J. Econ. Entomol. 58: 4-8.

Kido, H. and E. M. Stafford. 1966. Some effects of thiotepa on Drosophila melanogaster Meigen. J. Econ. Entomol. 59: 1064-1069.

Kilgore, W. W. and R. R. Painter. 1962. The effect of 5-fluorouracil on the viability of house fly eggs. J. Econ. Entomol. 55: 710-712.

Kilgore, W. W. and R. R. Painter. 1964. Effect of the chemosterilant apholate on the synthesis of cellular components in developing house fly eggs. Biochem. J. 92: 353-357.

Kilgore, W. W. and R. R. Painter. 1966. Insect chemosterilants: Incorporation of 5-fluorouracil into house fly eggs. J. Econ. Entomol. 59: 746-747.

Kissam, J. B. and S. B. Hays. 1966. Mortality and fertility response of Musca domestica adults to certain known mutagenic or anti-tumor agents. J. Econ. Entomol. 59: 748-749.

Knipling, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Entomol. 48: 459-462.

Knipling, E. F. 1959. Sterile male method of population control. Science. 130: 902-904.

Knipling, E. F. 1960. The eradication of the screw-worm fly. Sci. Amer. 203: 54-61.

Knipling, E. F. 1962. Potentialities and progress in the development of chemosterilants for insect control. J. Econ. Entomol. 55: 782-786.

Konecky, M. S. and N. Mitlin. 1955. Chemical impairment of development in house flies. J. Econ. Entomol. 48: 219-220.

LaBrecque, G. C. 1961. Studies with three alkylating agents as house fly sterilants. J. Econ. Entomol. 48: 684-689.

LaBrecque, G. C. 1963. Chemosterilants for the control of house flies. Adv. Chem. Ser. 41: 42-46.

LaBrecque, G. C., P. H. Adcock, and C. N. Smith. 1960. Tests with compounds affecting house fly metabolism. J. Econ. Entomol. 53: 802-805.

LaBrecque, G. C. and H. K. Gouck. 1963. Compounds affecting fertility in adult house flies. J. Econ. Entomol. 56: 476.

LaBrecque, G. C., D. W. Meifert, and R. L. Fye. 1963. A field study on the control of house flies with chemosterilant techniques. J. Econ. Entomol. 56: 150-152.

LaBrecque, G. C., D. W. Meifert, and C. N. Smith. 1962a. Mating competitiveness of chemosterilized and normal house flies. Science 136: 388-389.

LaBrecque, G. C., C. N. Smith, and D. W. Meifert. 1962b. A field experiment in the control of house flies with chemosterilant baits. J. Econ. Entomol. 55: 449-451.

LaChance, L. E. and M. M. Crystal. 1963. The modification of reproduction in insects treated with alkylating agents. II. Differential sensitivity oocyte meiotic stages to the induction of dominant lethals. Biol. Bull. 125: 280-288.

Ladd, T. L., Jr. 1966. Egg viability and longevity of Japanese beetles treated with tepa, apholate, and metepa. J. Econ. Entomol. 59: 422-425.

Lindquist, A. W. 1955. The use of gamma radiation of the screw-worm. J. Econ. Entomol. 48: 467-469.

Lindquist, D. A., L. J. Gorzycki, M. S. Mayer, A. L. Scales, and T. B. Davich. 1964. Laboratory studies on sterilization of the boll weevils with apholate. J. Econ. Entomol. 57: 745-750.

Mitlin, N. 1956. Inhibition of development in the house fly by 3,4-methylenedioxyphenyl compounds. J. Econ. Entomol. 49: 683-684.

Mitlin, N. and A. N. Baroody. 1958. The effect of some biologically active compounds on growth of house fly ovaries. J. Econ. Entomol. 51: 384-385.

Mitlin, N., B. A. Butt, and T. J. Shortino. 1957. Effect of mitotic poisons on house fly oviposition. Physiol. Zool. 133-136.

Mitlin, N., M. S. Konecky, and P. G. Piquett. 1954. The effect of a folic acid antagonist on the house fly. J. Econ. Entomol. 46: 932.

Morgan, P. B. and G. C. LaBrecque. 1962. The effect of apholate on the ovarian development of house flies. J. Econ. Entomol. 55: 626-628.

Morgan, P. B. and G. C. LaBrecque. 1964. Effect of tepa and metepa on ovarian development of house flies. J. Econ. Entomol. 57: 896-899.

Murvosh, C. M., G. C. LaBrecque, and C. N. Smith. 1964. Effect of three chemosterilants on house fly longevity and sterility. J. Econ. Entomol. 57: 89-93.

Nayar, J. K. 1963. Effect of synthetic "queen substance (9-oxodec-trans-2-enoic-acid) on ovary development of the house fly Musca domestica L. Nature 197: 923-924.

Ouye, M. T., H. M. Graham, R. S. Garcia, and D. F. Martin. 1965. Comparative mating competitiveness of metepa-sterilized and normal pink bollworm males in laboratory and field cages. J. Econ. Entomol. 58: 927-929.

Pain, J. 1961. Absence du pouvoir d'inhibition de le pheromone I - Sur le developpement ovarien des jeunes ouvrieres d'abeilles. Compt. Rend. Acad. Sci. Paris. 252: 2316-2317.

Painter, R. R. and W. W. Kilgore. 1964. Temporary and permanent sterilization of house flies with chemosterilants. J. Econ. Entomol. 57: 154-157.

Painter, R. R. and W. W. Kilgore. 1965. Chemosterilant effect of 5-fluoroorotic acid on house flies. J. Econ. Entomol. 58: 888-891.

Parish, J. C. and B. W. Arthur. 1965. Chemosterilization of house flies fed certain ethylenimine derivatives. J. Econ. Entomol. 58: 699-702.

Parish, J. C. and B. W. Arthur. 1965. Mammalian and insect metabolism of the chemosterilant thiotepa. J. Econ. Entomol. 58: 976-979.

Piquett, P. G. and J. C. Keller. 1962. A screening method for chemosterilants of the house fly. J. Econ. Entomol. 55: 261-262.

Plapp, F. W., Jr., W. S. Bigley, G. A. Chapman, and G. W. Eddy. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. J. Econ. Entomol. 55: 607-613.

Ristich, S. S., R. H. Ratcliffe, and D. Perlman. 1965. Chemosterilant properties, cytotoxicity, and mammalian toxicity of apholate and other P-N ring chemicals. J. Econ. Entomol. 58: 929-932.

Roach, S. H. and J. A. Buxton. 1965. Apholate and tepa as chemosterilants of the plum curculio. J. Econ. Entomol. 58: 802-803.

Robinson, A. G. 1959. Note on fecundity of the pea aphid, Acyrthosiphon pisum (Harris, caged on plants of broad bean, Vicia faba L., treated with various plant growth regulators. Can. Entomol. 91: 527-528.

Robinson, A. G. 1960. Effect of maleic hydrazide and other plant growth regulators on the pea aphid, Acyrthosiphon pisum (Harris) caged on broad bean, Vicia faba L. Can. Entomol. 92: 494-499.

Shaw, J. G. and M. S. Riviello. 1962. Sterility in the Mexican fruit fly caused by chemicals. Science 137: 754-755.

Shaw, J. G. and M. S. Riviello. 1965. Effectiveness of tepa-sterilized Mexican fruit flies released in mango grove. J. Econ. Entomol. 58: 26-28.

Shive, W. and C. G. Skinner. 1958. Metabolic antagonists. Ann. Rev. Biochem. 27: 643-678.

Simkover, H. G. 1964. 2-Imidazolidinone as an insect growth inhibitor and chemosterilant. J. Econ. Entomol. 57: 574-579.

Smith, C. N., G. C. LaBrecque, and A. B. Borkovec. 1964. Insect chemosterilants. Ann. Rev. Entomol. 9: 269-284.

Smith, F. F., A. L. Boswell, and T. J. Henneberry. 1965. Chemosterilant treatment of two greenhouse spider mites. J. Econ. Entomol. 58: 98-103.

Smith, P. K., M. V. Nadhkarni, E. G. Trams, and C. Davison. 1958. Distribution and fate of alkylating agents. Ann. New York. Acad. Sci. 68: 834-850.

Snedecor, G. W. 1961. Statistical methods. The Iowa State University Press, Ames, Iowa.

Tahori, A. S., A. H. Halevy, and G. Zeidler. 1965. Effect of some growth retardants on the oleander aphid, Aphis nerii (Boyer). J. Sci. Fd. Agric. 16: 568-569.

Uichanco, L. B. 1924. Studies on the embryogeny and post-natal development of Aphididae with special reference to the history of the "symbiotic organ or "mycetom". Philip. Jour. Sc. 24: 143-247.

Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature. 195: 786-787.

Weidhaas, D. E. and C. H. Schmidt. 1963. Mating ability of male mosquitoes, Aedes aegypti (L.), sterilized chemically or by gamma radiation. Mosquito News. 23: 32-34.

Weidhaas, D. E., C. H. Schmidt, and E. L. Seabrook. 1962. Field studies on the release of sterile males for the control of Anopheles quadrimaculatus. Mosquito News. 22: 283-291.

Woods, C. W., A. B. Borkovec, and F. M. Hart. 1964. Insect chemosterilants. I. N-Acylaziridines. J. Med. Chem. 7: 371-373.

Woolley, D. W. 1952. A study of antimetabolites. John Wiley and Sons, Inc., New York. pp. 1-261.

Young, J. R. and H. C. Cox. 1965. Evaluation of apholate and tepa as chemosterilants for the fall armyworm. J. Econ. Entomol. 58: 883-888.

Yule, W. N., E. V. Parups, and I. Hoffman. 1966. Toxicology of plant-translocated maleic hydrazide. Lack of effects on insect reproduction. J. Agr. Food. Chem. 14: 407-409.

Zukel, J. W. 1949-57, 1957-63. "A literature summary on Maleic Hydrizide", Naugatuck Chemical Division, U. S. Rubber Co., Bethany, Conn. 1949-1957, 1957-1963.