EFFECT OF SOME CHEMICAL SUBSTANCES ON THE FERTILITY OF *TETRANYCHUS URTICAE* KOCH

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CHAPTER I

INTRODUCTION

The two-spotted spider mite, Tetranychus urticae Koch (Acarina: Tetranychidae), is a particular pest in apple orchards and field crops in New Zealand. Due to the rapidity with which the organism develops resistance to insecticides, the populations become each year more difficult to control. Because of this problem, methods of population suppression other than by the application of pesticides are being explored. During investigations into the possibilities of using some sort of reproduction suppressant to control spider mite populations, it became clear that a considerable amount of research was needed to find new and different chemical compounds which would not only cause reproductive sterility in mite populations but could also be applied under field conditions.

As many of the recently reported insect reproduction suppressants, or 'chemosterilants' as they have become termed, are extremely toxic to vegetation as well as being highly poisonous to mammals, it was considered desirable to conduct the search on a broad basis and to ignore chemical groups such as the aziridines and triazines which, although they contain quite a few known chemosterilants of insects, are often phytotoxic and usually are highly poisonous to mammals.

Apart from pure curiosity to see how substances such as mammalian hormone derivatives, mitotic poisons like mercapto-ethanol, miticides and so forth affected reproduction in adult Tetranychids and their subsequent progeny, there was, as in all research on chemosterilants, a hope that something could be found to alter the reproductive potential of a naturally occurring population of mite or insect pests and thereby greatly reduce or even eradicate a population.

The impetus for research into reproduction suppressants mainly began with the eradication of the screw-worm fly, Cochliomyia hominovorax (Coquerel), from the island of Curacao which was accomplished in 1954 by liberating laboratory reared, sterilized, adult male flies by the million over the island (Baumhover et al., 1955). Each successive liberation of sterile males was carefully planned so that the numbers of sterile males would be about ten times that of the normal male flies resident on the island. Following a series of releases of the reared sterile flies, all of the native screw-worm flies disappeared from the island which, from all reports, is still free of this species.

In this classic experiment, sterility was induced by gamma irradiation of the pupae, a dose of about 5000 rads being found sufficient to sterilize both males and females (Bushland and

Hopkins, 1951, 1953). Because the production of radiation from X-ray generators or cobalt 60 sources involves much auxiliary shielding and equipment, use of radiation to sterilize insects in the field is severely limited. Treatment by chemosterilants therefore offers a more flexible means of rendering insects sterile.

Most reproduction suppressants are used for male sterilization, however, because of the reproductive characteristics of T. urticae, male sterility does not hold any great advantage over female sterilization. The Tetranychids reproduce by arrhenotoky, the process whereby males are produced from haploid unfertilised eggs and females from diploid fertilised eggs. If males only are sterilized, the female will therefore produce only male progeny and with the short life cycle of 7 to 12 days, the male progeny of the spider mites are available to mate with their parents so producing diploid eggs which result in females. Sterilization of the females has a dual effect in that the sterilized females will still mate with the males so reducing the number of effective matings carried out by each male, and the production of eggs and the hatchability thereof is also reduced.

The short life cycle is advantageous however, in that experiments on the chemical influences on reproduction can be rapidly conducted since, following the addition of the adult

female mite to the test substrate, egg production can be measured almost immediately and egg hatch can be recorded a few days after the cessation of egg laying. The present search for new chemosterilants of T. urticae therefore was concentrated on those chemicals affecting reproduction in the females.

CHAPTER II

REVIEW OF THE LITERATURE

INTRODUCTION

The use of chemicals as reproduction suppressants was first contemplated in early research into control of the screw-worm fly, but tests, carried out between 1947 and 1950, failed to reveal any chemicals possessing substantial sterilizing activity (LaChance et al., 1968). Radiation had earlier been shown to sterilize insects when Runner (1916) detected sterility in the cigarette beetle, Lasioderma serricorne F., after exposure to X-rays. Radiation, in the form of gamma-rays, was subsequently used to sterilize male screw-worm flies in the highly successful eradication programme in Curacao (Baumhover et al., 1955) and in south-eastern United States of America (Knipling, 1960a, b). With the successful use of radiation-induced sterility in population control, interest was revived in finding chemicals to use as sterilizing agents.

Chemical effects on reproduction were first noted in studies on mutagenesis in insects, but subsequent entomological work, directed towards the search for new sources of sterility, appeared to be conducted independent of the findings of the geneticists. In the genetic studies, the chemical induction of mutations was apparently first recorded when hereditary changes

were induced in several plants by injecting solutions of certain chemicals into ovaries immediately after fertilisation (MacDougal, 1911). Conclusive results were not however, obtained until about 1942 when Auerbach and Robson (1944, 1946) noted the mutagenic effect of nitrogen mustard (methyl bis(betachloroethyl) amine hydrochloride) on Drosophila melanogaster Meigen. The mutations resulted in the sterilization of males and this was subsequently confirmed by Demerec (1948) in studies with 1,2,5,6-dibenzthracene. Nitrogen mustard has been shown to have similar effects to X-rays in Drosophila in that mutation of the sperm results in a decrease in the proportion of viable eggs produced and a change in the sex ratio (Wallace, 1951). Nitrogen mustard has also caused complete sterility in Habrobracon juglandis Ashm, a parasitic wasp which reproduces by arrhenotoky (Whiting and Von Borstel, 1954), and "triazine" (2,4,6-tri(ethylenemine)-1,3,5-triazine) affected egg production in Drosophila when sperm was treated (Hersowitz, 1956).

In the entomological literature, one of the first recorded instances of chemical influence on reproduction was the effect of sub-lethal doses of pyrethum on the cigarette beetle causing the treated adults to deposit only half as many eggs as beetles not exposed to the spray (Tenhet, 1947). Results from attempts to induce sterility by the use of chemicals in Drosophila melanogaster (Goldsmith and Frank, 1952) and house flies (Ascher, 1957; Mitlin et al., 1954; Mitlin and Baroody, 1958) were sufficiently promising

to warrant further experimentation, and in the period 1958 to 1960, extensive tests to find chemicals inducing sterility in insects were initiated by the United States Department of Agriculture. Work by members of this Department resulted in large numbers of chemicals being found to induce sterility in the house fly (LaBrecque, 1962; LaBrecque and Gouck, 1963; LaBrecque et al., 1968) and particular attention was paid to a group of chemicals called alkylating agents (LaBrecque et al., 1960; LaBrecque, 1968). More recently many of the most effective chemosterilants have been tested on other insects such as the boll weevil (Klassen et al., 1968) and fruit flies (Keiser et al., 1965).

Preliminary field tests with the house fly (LaBrecque et al., 1962, 1963), using a chemosterilant-treated sugar bait, showed a good overall reduction of the population. Large-scale field tests were limited however, due to the extreme toxicity of many chemosterilants to warm-blooded animals (Hayes, 1964, 1968). Small-scale field tests on various insects have been undertaken (Weidhaas, 1968) using light or chemicals to entice the insects to the chemosterilant, but as yet, there have been no outstanding successes comparable to the screw-worm fly eradication.

It is to be hoped that the successful extension of the above small-scale field tests into large-scale situations may provide the impetus for further development of insect chemosterilization as a means of pest control.

CHEMOSTERILANTS

Many chemicals act as chemosterilants and these have been recorded by Ascher (1964), Smith et al. (1964), Borkovec (1966) and LaBrecque and Smith (1968). Different criteria are used by Borkovec (1966) and Giese (1968) to classify the chemicals possessing sterilizing activity (Table I). Borkovec (1966) uses a loose relationship between chemical activity and biological activity while Giese's (1968) classification is derived from the effect of the chemical on cellular processes. The three main classes proposed by Borkovec (1966) are referred to in most of the literature on chemosterilants and therefore will be used in this dissertation.

TABLE I: CLASSIFICATION OF CHEMOSTERILANTS

BORKOVEC (1966)	GIESE (1968)
1. Alkylating agents	1. Mitotic poisons
a) Aziridines	a) Alkylating agents
b) Nitrogen mustards	b) Antimetabolites
c) Sulphonic acid esters	i) Antibiotics
	ii) Nucleic acid inhibitors
2. Antimetabolites	iii) Specific metaphase inhibitors
3. Miscellaneous chemicals	c) Metabolic poisons
	2. Antimitotic agents

Alkylating agents

A number of chemosterilants can be described as alkylating agents and the main types are considered below.

a) Aziridines. The aziridines include the highly active chemosterilants tepa, metepa, tretamine and apholate, which have been extensively tested on a wide range of insects. Aziridinyl compounds have induced sterility in insects such as the house fly (LaBrecque et al., 1960; LaBrecque, 1961, 1962), Drosophila melanogaster (Hair and Adkins, 1967), the azuki bean weevil, Callosbruchus chinensis (Shinohara and Nagasawa, 1963), and the two-spotted spider mite, Tetranychus urticae (Smith et al., 1965). Some aziridines have caused female sterility, but action is primarily through sterilization of the male (Mason and Smith, 1967; Flint et al., 1968). The basic cellular effects however, of the aziridines is through their activity as 'aminoethylating' agents which react with nucleic acids and proteins, thus inhibiting cellular growth (Brooks and Lowley, 1961). Kilgore and Painter (1964) and Painter and Kilgore (1967) observed the inhibition of DNA activity and certain cellular enzymes in the eggs of apholate chemosterilized house flies and confirming this mode of action, Chamberlain and Barret (1968) found that apholate inhibits the incorporation of tritiated thymidine into ovarian DNA of the stable fly, Stomoxys calcitrans. Pillai and Agarwal (1969) reported that treatment with chemosterilants did not seem to alter RNA, DNA and protein content in the whole insect, therefore

suggesting that the activity of the aziridines is confined to germ cell nucleic acid and protein synthesis.

b) Nitrogen Mustards. The nitrogen mustards are prominent chemicals in cancer research as they possess considerable anti-tumour properties. The United States Cancer Chemotherapy National Service Centre (1963) lists 2,255 nitrogen mustards that have been tested as cancer therapeutants, but only a small proportion of these chemicals exhibited sterilizing activity in United States Department of Agriculture screening tests. The cellular effects of the nitrogen mustards are similar to those of the aziridines in that they function as 'aminoethylating' agents.

c) Sulphonic Acid Esters. The World Health Organisation (1962) reports sulphonic acid esters as cancer therapeutants. Chemicals from this group have been tested as chemosterilants (LaBrecque and Gouck, 1963; Flint et al., 1968) with varying success, but in general the high sterilizing properties of the aziridines have overshadowed the possible significance of other alkylating agents as chemosterilants.

Antimetabolites

Antimetabolites are inhibitors of nucleic acid synthesis (Borkovec, 1966) and specifically are structural analogues of purines, pyrimidines and folic acid. These chemicals are pre-

dominantly female sterilants. Borkovec (1966) lists many derivatives of purines and pyrimidines that have sterilizing properties, but only the fluorine containing derivatives seem worth of further study. 5-Fluorouracil and 5-fluorootic acid have been extensively tested on a wide range of insects, and Crystal (1963) found complete oviposition inhibition in screw-worm flies with 5-fluorouracil and 5-fluorootic acid; Bhalla and Robinson (1968) found that 5-fluorouracil induced permanent sterility in the pea aphid, Acyrthosiphon pisum (Harris); and Jalil and Morrison (1969a) reported that 5-fluorouracil caused sterility in T. urticae. Both of these fluorine derivatives are incorporated into preformed nucleic acids in spermatozoa or oocytes (Kilgore and Painter, 1966; Cline, 1968). Folic acid derivatives have been classed as specific metaphase inhibitors by Giese (1968) and contain two chemicals of promise, aminopterin and methotrexate, which have been shown to be highly active female sterilants in some species (LaBrecque et al., 1960; Hays and Cochran, 1964).

Miscellaneous Chemicals

Miscellaneous chemicals cover a wide range of chemical structure and activity. Derivatives of non-alkylating s-triazines are analogous to the pteridine part of the folic acid molecule but act primarily as male sterilants, so Borkovec (1966) therefore considers these agents under the heading of

"miscellaneous chemicals". The chemosterilants, hempa and hemel, are structurally analogous to tepa and tretamine, but lack the alkylating properties of the aziridines (Chang et al., 1964).

Triphenyltin compounds form another important group of chemosterilants (Borkovec, 1966). Sterilization of adult houseflies with this group of chemicals occurred well below the lethal concentration (Kenaga, 1965) and the most active compounds were the triphenyltin hydroxides, allyl triphenyltin and bis(triphenyltin sulphide). Females were sterilized at a lower concentration than males.

Antibiotics, such as Cycloheximide and Actinomycin, have been tested on a range of insects, and have shown considerable potential as sterilizing agents. Kohls et al. (1966) found that some antibiotics inhibited egg development in houseflies, and antibiotics have induced sterility in the two-spotted mite, Tetranychus urticae (Harries, 1961, 1963, 1968; Jeppson et al., 1966).

Antimitotic agents, which inhibit the formation or breakdown of the mitotic apparatus, have been found to have sterilizing properties. Colchicine has sterilized insects such as the screwworm fly (Chamberlain and Hopkin, 1960), fruit flies (Keiser et al., 1965), and houseflies (Gouck and LaBrecque, 1964).

Other chemical compounds which have shown sterilizing properties are: derivatives of urea and thiourea (Mitlin and Baroody, 1958), Queen substance (Nayar, 1963), a vegetable oil from Sterculia foetida (Beroza and LaBrecque, 1967), mammalian hormones such as m-xylohydroquinone (Ascher and Hirsch, 1963) and some known insecticides, the activity of which has been reviewed by Ascher (1964).

INDUCTION OF STERILITY

Sterility, or the suppression of reproductive processes, may be attained by various means: dominant lethal mutations in the reproductive cells of both sexes, inhibition of egg production, disruption of spermatogenesis and sperm inactivation. In the present study, interest is largely confined to those processes directly affecting reproduction in the female although the disruption of spermatogenesis and sperm inactivation in the male are worthy of consideration.

Dominant Lethal Mutations

Dominant lethal mutations were first observed in amphibian sperm following irradiation (Hertwig, 1911, cf. LaChance, 1967), and in insects, Muller (1927) detected mutagenic effects of radiation which were characterised as 'dominant lethal mutations'. Sonnenblick and Henshaw (1941) defined dominant lethal mutations as:

'nuclear alterations which can affect the death of the zygote even though they are present in single doses, that is introduced by but one of the germ cells which unite at fertilisation'.

In essence, lethal mutations are not lethal to the treated cell but to its descendants - the zygote it forms.

Many chemicals are known to produce dominant lethal mutations (LaChance et al., 1968), but early insect studies were limited to work on chromosome translocation in Drosophila (Auerbach, 1951, 1958). Studies on the production of chromosomal abberations by chemosterilants have been limited (LaChance and Riemann, 1964; Murray and Bickley, 1964; LaChance and Leverich, 1968), but the information is sufficient to show that alkylating agents and chemicals directly related to nucleic acid and protein metabolism are the principal chemosterilants causing dominant lethal mutations.

Disruption of Spermatogenesis

The condition where differentiated sperm are not produced or the supply becomes exhausted, has been described by U.S.D.A. workers as 'aspermia' (LaChance, 1967). Little is known about the disruption of spermatogenesis by chemosterilants, but work by LaChance and Crystal (1963) on the screw-worm showed differential germinal cell sensitivity to chemosterilants. Cantwell and Henneberry (1963) observed the cessation of spermatogenesis

in the anterior portions of the testes of Drosophila melanogaster when fed apholate for 24 hours and likewise, LaChance et al. (1969) reported the death of all gonial cells in the testes of the house fly treated with some aziridines.

Sperm Inactivation

Sterility induced by sperm inactivation is manifested by way of a loss of motility or fertilising capacity of the treated sperm. Sperm inactivation is commonly associated with dominant lethality, and is usually encountered after administration of high doses of radiation (Henneberry, 1964) or chemicals (LaChance, 1967). Chemical studies have shown that sperm inactivation occurred in Bracon hebetor Say (Hymenoptera: Braconidae) following the induction of dominant lethality by treatment with several aziridines (LaChance and Leverich, 1968). This effect is not common to all chemosterilants however, as tepa and its analogues will induce significant sperm inactivation even at substerilizing doses (LaChance, 1966).

Infecundity

Considerable confusion surrounds the use of the term 'fecundity'. It was originally used to indicate the production of gametes or eggs by females (Norris, 1934), but now often refers to the number of progeny produced. In the present study, sterility via the female is regarded as being manifested by a reduction in eggs laid and a reduced egg hatch. Fecundity

therefore indicates the number of fertile eggs produced by the female.

The inhibition of egg production differs from the induction of dominant lethality in that the processes of oogenesis can be interrupted by external agents, such as chemicals and radiation, inducing gonial cell death or damage to nutritive cells (LaChance et al., 1968). Dominant lethal mutations are, however, primarily responsible for the failure of eggs to hatch.

Oogenesis has been inhibited by a considerable number of chemicals, most being antimetabolites and alkylating agents. Gouck and LaBrecque (1964) found 27 chemicals that inhibited oogenesis in the house fly, the majority being alkylating agents. Mitlin and Baroody (1958) listed 15 chemicals that reduced the fecundity of the house fly, and other chemicals affecting egg production have been recorded by Crystal (1963), Ascher (1964) and LaChance et al. (1968).

CHEMOSTERILIZATION OF SPIDER MITES

Sterilization of spider mites has received comparatively little attention from research workers. Although sterility has been induced in males and females of T. urticae following exposure to gamma radiation (Henneberry, 1964), most of the work has been concentrated on chemical reproduction suppressants and the first chemical effects were noted with the insecticide

Tedion (p-chlorophenyl 2,4,5-trichlorophenyl sulphone), which killed the eggs, larvae and nymphs of spider mites and caused the females to lay infertile eggs (Flik, 1955; Baath and Davidson, 1959). Chemosterilant testing on spider mites, in common with tests on other insects, has subsequently become concentrated on alkylating agents and antimetabolites. Cressman (1965) found that the alkylating agents, tepa, apholate and aphamide, all reduced egg laying and egg hatch, and induced male sterility. Smith et al. (1965) obtained similar results in extensive testing of apholate on males and females of T. urticae, and Jalil and Morrison (1969a) found the alkylating agents, apholate and tepa, to sterilize both males and females, while the antimetabolites, 5-fluorouracil and 5-fluorootic acid, sterilized the females. Tests on populations of T. urticae (Jalil and Morrison, 1969d) indicated that apholate is more effective than 5-fluorouracil at population suppression, although the advantage of the male sterilant, apholate, is not as great as would be expected with treatment of an insect population which reproduces normally.

Certain antibiotics have been found to sterilize spider mites. Harries (1961, 1963, 1965) and Jeppson et al. (1966) have reported a significant inhibition of egg laying by Cycloheximide, and a similar effect has been found with Actinomycin D (Harries, 1968). Egg lay has also been reduced by some of the

metal chelate plant growth additives, iron and magnesium chelates being the most effective (Terriere and Rajadhyaksha, 1964).

BIOLOGY AND BEHAVIOUR OF TETRANYCHUS URTICAE

The life cycle of the two-spotted spider mite is described in detail by Cagle (1949). The stages in the life cycle are the egg, larva, nymphochrysalis, protonymph, deutochrysalis, deutonymph, teleochrysalis and adult. The eggs are spherical, approximately 0.14 mm in diameter, and are whitish at first, yellowing as development proceeds, with the two carmine eyespots becoming evident. The incubation period is three days on average, at a temperature of 30°C (Helle, 1962). Boudreaux (1958) reported that egg hatch varied little between extremes of humidity (95% and 35% RH), but in contrast, Harrison and Smith (1960, 1961) found that optimum egg hatch occurred between 50% and 90% RH and at 100% RH no eggs hatched.

The emerging larvae have six legs and are about the size and shape of the egg. A quiescent stage, the nymphochrysalis, is followed by the eight-legged protonymph. Body colour darkens as development proceeds, with two black spots appearing from the presence of ingested material in the digestive tract (Cagle, 1949). Growth proceeds with two resting stages, the deutochrysalis and the teleochrysalis, intervening between the protonymph, deutonymph and the adult. The male becomes readily distinguishable as an adult, being much smaller

than the adult female. The male is characteristically pear-shaped, and is more active and of a lighter colour than the female. During maturation of the adult female the two black spots increase in size until black becomes the predominant colour (see Plate 1).

The duration of the life cycle is correlated to environmental temperature. In the temperature range of 23.9 to 29.4°C, the life cycle is completed in 7 to 12 days (Boudreaux, 1963). Helle (1962) reported that at a temperature of 30°C, development from egg laying to emergence of the adult female took 7 days, while Ballantyne (1966) found that development at 29°C took 8 days - 4 days incubation and 4 days of post-embryonic development.

An understanding of the development of the reproductive organs and the mating processes of spider mites is fundamental to this study. Blauvelt (1945) and Beament (1951) studied the anatomy of the reproductive system of adult Tetranychids but little work was done on the development of reproductive organs in immature stages of T. urticae. Jalil (1969) and Jalil and Morrison (1969) have recently followed the changes in internal morphology of T. urticae through five development stages. Ovarian development does not begin until the mite reaches the deutonymphal stage, and during the teleochrysalis stage the cells in the triangular ganglionic and ovarian mass of cells separate with development being completed in 24 to 48 hours. The germarium of newly emerged females contains a few mature oocytes and ovi-

Photo: E.R. Mangin

PLATE 1: Part of a Population of Tetranychus urticae on a Bean Leaf.

KEY: M - Male, MF - Mature Adult Female, IF - Immature Adult Female, E - Eggs.

position begins after a short preoviposition period. The oviposition rate reaches a peak in T. urticae between the 6th and 9th days of the oviposition period (Lehr and Smith, 1957). Egg production may vary depending on strains and environmental conditions, but in the present series of experiments, a daily oviposition rate of 11 to 13 eggs was found in the untreated checks.

The testes first appear in the male in the late deutonymphal stage and develop during the teleochrysalis stage (Jalil and Morrison, 1969). The adult male generally emerges before the female (Helle, 1962) and the males can be seen standing guard over the female teleochrysalii. On emergence of the female, copulation occurs by the male crawling beneath the body of the female, raising the tip of the abdomen and inserting the aedegus into the genital opening of the female. Although copulation may occur several times, Cagle (1949) and Helle (1962) report that generally one single fertilisation will permit a female to produce diploid eggs for the rest of her life.

Sex determination in the Tetranychids appears to be almost entirely by arrhenotoky (Huffaker et al., 1969). Arrhenotoky is a form of parthenogenesis in which the haploid, unfertilised eggs produce males and the diploid eggs produce females (White, 1954). Male haploidy in spider mites was first demonstrated by Schrader (1923, cf White, 1954). Unmated females

produce only haploid eggs and mated females deposit a mixture of haploid and diploid eggs. Boudreaux (1963) considers the sex ratio of spider mite offspring to be largely a factor of the amount of spermatozoa introduced during the mating act, but Overmeer (1967) found a rather fixed sex ratio that fluctuates around a mean. Control of sex ratios in T. urticae has now been found to be dependent on the phenotype of the mother (Overmeer and Harrison, 1969).

The ability of unfertilised females to produce male progeny is of great importance in the search for new chemosterilants of spider mites. Longevity studies have shown that adults of T. urticae will live for approximately 40 days under controlled conditions (Jalil and Morrison, 1969a). If males alone are sterilised, the subsequently unfertilised females will produce haploid male progeny. Allowing for a development period of 7 to 12 days, the male offspring will be available to mate with their parents, thus providing a new source of females. In contrast to many insect species, male sterility of spider mites would not hold a great advantage over female sterilisation in population control. The emphasis in the present series of experiments has therefore been directed towards the search for chemosterilants reducing the fecundity of the female.

Other biological factors which have considerable influence in any programme utilising chemicals for control of reproduction are diapause characteristics and the methods of dispersal of the organisms. Diapause or arrested development in T. urticae is photoperiodicity modified by temperature and nutrition. In most Tetranychids, a combination of a reduction in daily photoperiod and decreasing average temperature induces young adult females to enter a diapause state (Lees, 1953b). Helle (1962) investigated the causes of diapause in T. urticae and found that photoperiod followed by lowering temperatures was the predominant controlling factor, while lowered nutritional status may also induce diapause (Lees, 1953a). Diapausing females in the genus Tetranychus are recognisable by their pellucid orange-red colour (Boudreaux, 1963), and the resultant cessation of egg production has meant that the identification of diapause females of T. urticae was crucial to these experiments.

Dispersal of spider mite populations occurs during the preoviposition period, during which Hussey and Parr (1963) observed movements of young adults of T. urticae to the higher, younger leaves of the host plant. Under overcrowded conditions, Hussey and Parr (1963) also reported migration between host plants by the formation of "ropes" and the movement of these ropes by slight air currents. Fleschner et al. (1958) failed to observe any spinning of threads and drifting by T. urticae

but the author has observed the formation of agitated masses of mites and the spinning of silken threads in populations of T. urticae held at Lincoln College. Air currents may cause some individuals to drop to the ground. Movements on the ground and on the host plant show a strong positive response to light during the preoviposition period and periods of desiccation and shortage of food (Suski and Naegele, 1963).

CHAPTER III

METHODS AND MATERIALS

SPIDER MITE TAXONOMY AND STRAINS

Taxonomy

Considerable confusion has existed over the correct taxonomic designation for the two-spotted spider mites. Harrison (1962) considered the New Zealand form to be *Tetranychus telarius* (Linnaeus), but results of cross breeding experiments within the *T. telarius* complex indicate that *Tetranychus urticae* Koch is the correct name for the green two-spotted spider mite (Boudreaux and Dosse, 1963; Tuttle and Baker, 1968). Crossing experiments with New Zealand and German strains (Ballantyne, 1966) confirm this designation. The two-spotted spider mites used in the present study will thus be called *T. urticae*.

Strains

Only one strain of *T. urticae* has been used in the present work. It is designated the LINCOLN NORMAL (LN) strain and was collected from bean plants at Lincoln College in 1965. Most of the chemicals tested in the present work were organophosphates, so basic biological testing was confined to this one strain which reacts normally with organophosphate insect-

icides. Future tests of potential chemosterilants of spider mites could be extended by the use of strains resistant to these insecticides.

MAINTENANCE, ISOLATION AND DISPOSAL OF STRAINS

The LN strain used during this work was reared on the French Dwarf bean, *Phaseolus vulgaris*. The colony was maintained in a glasshouse unit having heating and cooling equipment which kept an average temperature of 29°C (range 26-32°C) and a relative humidity of 50-80%. White wash on the external surface of the glass was used in the summer months as a subsidiary temperature control. During the winter, artificial lighting was used to give a photoperiod of 16 hours and so reduce the incidence of the pellucid-orange coloured diapausing females.

Host plants were grown in 12 cm pots which were bottom-watered daily via a 17 cm dish. The whole unit was kept in a galvanized zinc water tray (40 x 80 cm) which acted as the primary barrier against dispersal. The trays were held in plastic-walled cages which were used to prevent the contamination of other colonies by mites carried in air currents (Plate 2). Water was flowing across the floor of the glasshouse and, should any individual circumvent the first two barriers, this film of water acted as a final impediment to dispersal.

PLATE 2: Barriers Against Contamination of Strains: Infested Bean Plants Held in a Water-filled Tray and Enclosed by a Plastic-walled Cage.

Once the host plants showed evidence of overcrowding, the bean plants were cut off at the base and immediately put in polythene bags for later incineration. Portions of leaves from the old colony were placed on the primary leaves of the new plants to initiate new colonies.

CHEMICAL SCREENING METHODS

Chemosterilant screening methods are similar to those now in use for insecticide screenings (LaBrecque, 1968). Acaricidal testing procedures therefore form the basis for the development of screening methods for potential chemosterilants of spider mites.

Contact acaricidal action can be tested in several ways.

a) Topical application. Harrison (1961) describes a technique in which a measured amount of insecticide is topically applied to mites or small insects. Ballantyne (1966) and Thiele (1967) used this method in acaricidal tests on T. urticae. Following treatment the mites are often confined in leaf cages (Tashiro, 1967), or in slide cages (Voss, 1963).

b) Slide-dip Method. A piece of double-sided Scotch tape is attached to a glass slide and adult mites are fixed to the tape by the dorsal part of the hysterosoma (Dittrich, 1962). The slide is dipped in the toxicant for five seconds then held

under constant conditions for 24 hours when the mortality is recorded.

c) Contact on a leaf. The acaricide may be applied by dipping whole leaves (Lippold, 1961), or spraying whole plants caged on a revolving turntable (Mailloux and Morrison, 1962). Residues can be applied to leaves with greater accuracy by use of the Potter's tower where known quantities of acaricide can be applied (Lee, 1967).

Dittrich (1962) compared the cage-spray, the slide-dip and leaf-dip methods of testing acaricides. Cage-spraying gave the least variability at intermediate sensitivity, the slide-dip gave greater variability and the highest sensitivity, and leaf-dipping gave the greatest variability with the least sensitivity. Ballantyne (1966) found the greatest accuracy with the topical application method, but while this technique is especially suitable for resistance studies, its use as a screening method is restricted owing to the limited range of solvents which can be used. Smith (1968) compared a direct-dosing technique with the leaf-dip and glass-slide residue methods. No clear differences in mortality were found, although some individuals in the leaf-dip test may avoid the residues by spending long periods on their mat of webbing away from the leaf surface.

The selection of suitable screening methods for chemosterilants is restricted by the need to keep the insects or mites alive in conditions that will not affect normal reproduction. Gouck et al. (1963) compared the techniques used for screening chemosterilants in the U.S.D.A. tests on house flies and screw-worm flies, and found that incorporating the chemosterilants in the diet gave more reliable results than either topical application or larval dipping. Tests on the chemosterilization of spider mites (Chapter 2) have either treated the substratum (generally bean leaves) and then added the mites, or have treated the colonies by some contact method, and then added the treated individuals onto a host plant.

The separation of contact and feeding effects of spider mite chemosterilants is most difficult. The only method for applying a measured dose of chemical to each individual is by topical application (Harrison, 1961). Apart from the limited range of solvents which can be used, the mechanical difficulties in applying a very small drop of the test solution onto the abdomen of the mite without the chemical reaching the mouth region, precludes the use of topical application techniques in general chemosterilant screening trials with spider mites. The feeding of insects possessing sucking and piercing mouthparts through an artificial membrane would be a useful addition to the screening methods at present available. This method

has been used in aphid tests (Bhalla and Robinson, 1968), but only recently has an artificial diet been formulated for spider mites (Walling et al., 1968). Until the diet can be readily prepared, and the full effects on spider mite biology are known, this technique cannot be added to the range of screening methods at present used. Mode of action of the chemicals used in this study must therefore be empirically derived.

CHEMOSTERILANT SCREENING METHODS

Prior to intensive biological testing of candidate chemosterilants, preliminary experiments were conducted to discern any mortality or sterility effects, and to determine an optimum concentration range. Whole primary leaves of the French dwarf bean were dipped in the test solution and maintained on cotton wool as described by Seigler (1947). Twenty adult females of approximately the same size were selected direct from the stock colony and placed on the leaf surface for 24 to 48 hours. An untreated check was run for each experiment. Eggs laid were recorded and the adult female mites removed. Egg hatch was observed five days later.

On the basis of results from these preliminary tests, certain chemicals were selected for intensive testing of their sterilizing properties. For these tests, leaf discs, 11 mm

in diameter, were cut by cork borer from bean leaves, dipped in the test solution for 20 seconds, and placed on blotting paper in clear plastic trays (40 cm long x 32 cm wide x 4.5 cm deep). When the discs were dry, one adult female mite was added to each disc. 15 discs were used per treatment. The mites were maintained on the discs for 48 hours, after which the eggs laid were recorded. The adult mites were removed and egg hatch was recorded 5 days later.

Contamination of the untreated checks from surplus chemicals on the treated discs, necessitated a change in testing procedure. The same sized discs were used but were placed on blotting paper discs, 9 cm in diameter. The blotting paper discs and the leaf discs together were dipped in the test solution and then placed on a cotton wool filled plastic petri dish (Plate 3). Each petri dish half had several holes in its base for the purpose of bottom-watering. The petri dishes were placed in the clear plastic trays and a self-watering device, consisting of a plastic bottle with a short polythene tube through its base, ensured a stable water level. The test trays were maintained in controlled conditions of 26.5°C, 50-60% relative humidity and 24 hours light.

Mites were handled with a fine sable-haired brush, slightly moistened to produce a fine tip. A low power

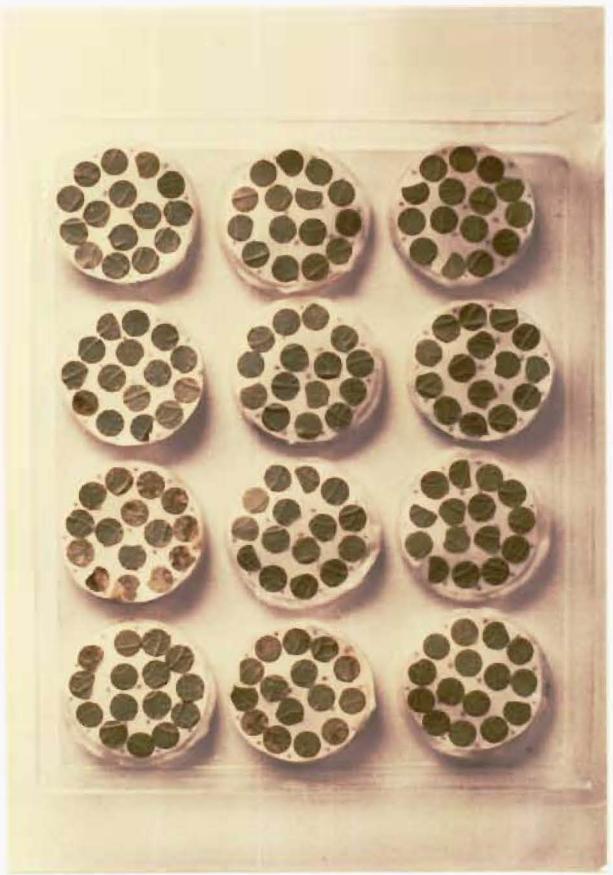

PLATE 3: Treated Leaf Discs on Blotting Paper and held in a Perspex Tray.

binocular microscope, 12X magnification, was used when transferring the mites.

Experiments for intensive biological testing were of a completely randomized block design. Two concentrations plus an untreated check were generally used, and each treatment was replicated 10 times. In the intial treatments, 15 discs were treated per concentration to allow for mortalities of mites due to handling or drowning on the surrounding wet blotting paper. If more than 10 mites were still alive after 48 hours, the required number of replicates were obtained by randomly eliminating discs. This step was necessary since the computer programme for the analysis of the results did not allow for missing data.

CHEMICALS

Preliminary experiments encompassed a wide range of chemicals. Agents tested included the following:-

'Plictran' (tributyltin acetate), a miticide

5-Fluorouracil

'Estratrein - 1,3,5(10)-estratrien-3,17β diol-3-methyl ether

'Pregradione' - 4,16-Pregnadien-3,20-dione

Cycloheximide - 3- 2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl -glutarimide

2,4-bis-dimethylamino-6, dimethoxyphosphinothioylthio-1, 3,5-triazine

Mercaptoethanol

Propylene oxide

9-methyl-8-azaxanthine and 8-azaxanthine

Trimethyl phosphate, a trialkyl phosphate.

On the basis of the preliminary results, intensive testing was confined to the trialkyl phosphate group, which is known to function as alkylating agents (Jones et al., 1966). The following chemicals, either within this group or chemical relatives, were tested:

Trimethyl phosphate

Triethy phosphate

Tri-n-butyl phosphate

Tri-isobutyl phosphate

Triallyl phosphate

Triphenyl phosphate

Trimethyl thiophosphate

Sodium dimethylphosphate

Diethyl phosphite

Dibutyl phosphite

Diphenyl methylphosphate

Diethyl benzylphosphonate

Diethyl cyanomethylphosphonate.

All the above chemicals were tested as for preliminary screenings and only chemicals showing some degree of activity were used in intensive experimentation. Chemicals found to be

moderately to severely phytotoxic to the bean leaves, were also discarded from further testing.

Since the large majority of the chemicals were liquids, pipettes were used to measure the require quantities. Prior to preparation of the test solution, solubility tests were carried out. Those chemicals which were not soluble in water, dissolved in a 10% solution of ethyl alcohol. Concentrations used generally ranged between 2.5% and 0.5%, but the final concentrations selected depended on the preliminary results. 50 to 100 ml of each solution were prepared, and prior to use, one drop of detergent per 50 ml was added to act as a wetting agent.

CHAPTER IV

RESULTS AND DISCUSSION

PRELIMINARY SCREENING RESULTS

Preliminary tests were carried out on a range of chemical compounds. Some of the chemicals selected for testing, for example Cycloheximide and 5-fluorouracil, had already been reported as showing insect sterilant activity. Others were known to be alkylating agents or were related to known antimetabolites or hormones. The results of these preliminary tests are presented in Table II. These results were not statistically analysed since the experiments were not replicated.

The miticide, 'Plictran', failed to affect fertility and, contrary to the results reported by Jalil and Morrison (1969a,b,d), no sterility was induced by the antimetabolite, 5-fluorouracil. The steroids, 'Estratrien', and 'Pregnadione', were tested and 'Pregnadione', which is a chemical precursor of the mammalian hormone, Progesterone, reduced egg lay. The antibiotic, Cycloheximide, reduced the number of eggs laid, confirming the early results of Harries (1961, 1963), but the severe phytotoxicity of this chemical ruled out further tests on bean leaves. The phosphorylated triazine, 2,4-bis-dimethylamino-6-dimethoxyphosphino-thioylthio-1, 3,5-triazine, reduced

TABLE II

STERILITY EFFECTS OF CHEMICALS TESTED IN PRELIMINARY SCREEN

CHEMICAL	CONCENTRATION	REDUCTION IN EGGS LAID	REDUCTION IN EGGS HATCHED
Plictran	125-0.5 ppm	X	X
5-Fluorouracil	0.25%	X	X
Estratrien	1.0%	X	X
Pregnadien	1.0%	✓	X
Cycloheximide	200-100 ppm	✓p	✓p
Phosphorylated triazine+	1.0%	✓	✓
Mercaptoethanol	1.6%	X p	X p
Propylene oxide	1.6%	X	X
9-Me-8-azaxanthine	0.5%	X	X
8-azaxanthine	1.0%	✓	X
Trimethyl phosphate	1.5%	✓	✓

p - Phytotoxic at concentrations used.

X - No effect.

✓ - Some effect.

\+ - 2,4-bis-diethylamino-6, dimethoxyphosphinothioylthio-1,3,5-triazine.

egg lay and egg hatch, but in the time available this chemical could not be subject to further investigation. Mercaptoethanol is a known inhibitor of cell division (Giese, 1968) but showed no sterilizing activity in the present tests. Of the group of chemicals comprising propylene oxide, 9-Me-8-azaxanthine and 8-azaxanthine, only the last showed activity, reducing the number of eggs laid. Trimethyl phosphate considerably reduced both eggs laid and eggs hatched. The preliminary work also indicated that trimethyl phosphate had a low toxicity and lacked phytotoxicity. The trialkyl phosphate group was therefore chosen for further investigation and the results from the initial studies into the sterility effects of trimethyl phosphate and triethyl phosphate have been published elsewhere (Osborne et al., 1969). The tests have since been extended to encompass a wide range of trialkyl phosphates.

STERILITY TESTS ON TRIALKYL PHOSPHATES AND CHEMICAL RELATIVES

Prior to intensive biological testing of the trialkyl phosphates and related compounds, tests were conducted to determine the approximate level of activity and the optimum concentration for the testing of each chemical. The full range of chemicals tested in the preliminary experiments, together with indications of sterilising activity, is shown in Table III.

TABLE III

PRELIMINARY SCREENING OF TRIALKYL PHOSPHATES

AND CHEMICAL RELATIVES

CHEMICAL	CONCENTRATION	STERILITY EFFECT	
		REDUCTION IN EGGS LAID	REDUCTION IN EGGS HATCHED
Trimethyl phosphate	2.5 - 1.5%	✓	✓
Triethyl phosphate	2.0 - 1.0%	✓	✓
Tri-n-butyl phosphate	0.2 - 0.1%	X d p	X
Tri-isobutyl phosphate	0.2 - 0.1%	X r p	X
Triallyl phosphate	0.2 - 0.1%	X	X
Triphenyl phosphate	0.2 - 0.1%	X	X
Trimethyl thiophosphate	0.1 - 0.025%	✓	X
Sodium dimethylphosphate	0.38 - 0.19%	X	X
Diethyl phosphite	2.5%	X p	X
Dibutyl phosphite	0.5 - 0.125%	X	X
Diphenyl methylphosphate	1.5 - 0.75%	X m p	✓
Dimethyl methylphosphonate	7.5 - 1.5%	✓	✓
Diethyl benzylphosphonate	2.0 - 1.0%	X d p	X
Diethyl cyanomethyl-phosphonate	2.0 - 1.0%	✓	✓

p - Phytotoxic at concentrations used.

d - Mortality caused by the chemical.

r - Repellent effect of chemical.

m - Marginal effect.

X - No effect.

✓ - Some effect.

Within the trialkyl phosphate group, substantial sterilizing activity was found only with trimethyl phosphate and triethyl phosphate. Tri-n-butyl phosphate caused high mortality at the concentrations used, while tri-isobutyl phosphate acted as a strong repellent to T. urticae. Trimethyl thiophosphate reduced egg lay but did not affect egg hatch.

The concentrations selected for each chemical in the sterility tests were based on the results obtained from the preliminary experiments. The initial concentrations used were between 2.0 and 3.0 per cent. If mortality and/or severe phytotoxicity was recorded, the concentrations were reduced until the majority of mites remained alive during testing and the test discs showed little evidence of phytotoxicity. The absence of mortality combined with normal egg production indicated a lack of sterilizing properties and the chemical was therefore removed from further testing.

Trialkyl phosphates used in the tests were commercial samples obtained from Aldrich Chemical Company, or Hopkins and Williams Limited. Some initial variation in results was recorded when trimethyl phosphate from different suppliers was used. Paper chromatographic analysis revealed the presence of some dimethyl phosphate in all samples. This product, which is readily formed from trimethyl phosphate on hydrolysis, possesses no sterilizing activity (Table III).

Trimethyl phosphate ($(MeO)_3P=O$) may be attacked by chemical nucleophilic agents in two different ways. Hydroxide ions (OH^-) attack at the phosphorus atoms. Other reagents, including amines, appear to attack preferentially at the carbon of an alkyl group. In this latter case, trimethyl phosphate is functioning as an alkylating agent. It was because of the facility with which trimethyl phosphate undergoes reactions of this latter type (Jones et al., 1966) that it was included in the present tests.

On examining Table III it will be observed that phosphate triesters, which contain no alkyl groups (e.g. triphenyl phosphate) are inactive. In fact, triesters which contain no methyl or ethyl groups are inactive although the situation is complicated by the acaricidal activity of tri-n-butyl phosphate and the repellent activity of tri-isobutyl phosphate. If, as might be supposed, leaf penetration is a factor influencing activity, the relatively low water solubility of the higher esters must also be taken into account. It should be noted however, that two esters of phosphorus acid, diethyl phosphite and dibutyl phosphite, both poor alkylating agents, are inactive as sterilants.

Relatives of the trialkyl phosphates which might be expected to function as alkylating agents, were also tested for sterility effects. In the case of diphenyl methylphosphonate and of diethyl benzylphosphonate, severe phytotoxicity

was encountered. Dimethyl methylphosphonate and diethyl cyanomethylphosphonate were active sterilants.

On the basis of the results in Table III, five chemicals were selected for intensive biological testing:

Trimethyl phosphate

Triethyl phosphate

Trimethyl thiophosphate

Dimethyl methylphosphonate

Diethyl cyanomethylphosphonate.

Wherever possible, statistical analysis of the experimental results was carried out on an IBM 1130 computer installed at Lincoln College. Duncan's Multiple Range Test (Duncan, 1955) was used to compare treatment means for significant differences and in the following tables, those treatments with no common letter are significantly different at the 5 per cent level ($P = 0.05$). In the complete analysis presented in the appendix, the results of the tests for significance at the 1 per cent level ($P = 0.01$) are also included. All experiments also underwent an analysis of variance and F-test (Snedecor, 1956) as a subsidiary test for significance (see appendix). It was not possible to analyse directly eggs hatched for treatment effects since, in most cases, the numbers of eggs laid were already reduced by the effect of the chemical. Egg hatch was therefore analysed on the basis of percentage hatch of eggs on

each leaf disc. The low numbers of eggs laid in some treatments gave rise to considerable variations in the percentage hatch between discs. The results of these experiments are given in Table IV.

The results of Table IV suggest a correlation between chemical structure and chemosterilant activity. Thus dimethyl methylphosphonate, which is a somewhat less effective alkylating agent than trimethyl phosphate, is also less effective in reducing numbers of eggs laid and hatched. Diethyl cyanomethylphosphonate, which is likely to be more effective as an alkylating agent than trimethyl phosphate, is more effective as a chemosterilant. It would be of interest to pursue this line of reasoning further using a larger number of chemicals.

The results obtained with trimethyl thiophosphate are of interest. The number of eggs laid is reduced by the percentage hatch is unaltered which suggests that different chemical reactions may be involved in the inhibition of these two processes.

TABLE IV

SUMMARY OF STERILITY EFFECTS OF THE TRIALKYL PHOSPHATES AND CHEMICAL RELATIVES

TREATMENT	CONCENTRATION	STERILITY EFFECT		
		EGGS LAID*	EGGS HATCHED*	% HATCH*
Trimethyl phosphate	Control	27.3 a	25.9	95.5 a
	1.5%	19.1 b	13.8	67.2 b
	2.5%	10.8 c	3.3	21.3 c
Triethyl phosphate	Control	22.0 a	21.3	96.9 a
	1.5%	14.1 b	13.2	93.9 a
	2.5%	8.0 c	5.3	66.1 b
Trimethyl thiophosphate	Control	21.4 a	20.7	96.8 a
	0.05%	13.7 b	12.9	88.7 a
	0.025%	6.1(2d) c	5.7	96.3 a
Dimethyl methyl-phosphonate	Control	25.7 a	24.1	94.0 a
	1.5%	18.3 a	12.1	60.4 b
	2.5%	19.6 a	10.3	44.9 b
	7.5%	6.6 b	3.6	66.3
Diethyl cyanomethyl-phosphonate	Control	27.3 a	26.8	98.4 a
	1.0%	13.1 b	11.8	80.1 a
	2.0%	3.9 c	1.8	46.6 b

* - Mean of 10 replicates. d - Mites dead due to chemical.

PERMANENCY OF STERILITY

To establish the permanency of sterility induced by the two chemosterilants, triethyl phosphate and diethyl cyanomethylphosphonate, two concentrations, 2.0 and 1.0 per cent of each chemical were tested. The testing methods were as described in Chapter 3. Adult female mites were maintained on treated discs for 48 hours after which they were removed and placed on untreated discs for a further 48 hours. Eggs laid and eggs hatched were recorded after the interval on the treated and untreated discs.

The initial treatments induced some degree of sterility with all chemicals and concentrations and significant differences were shown at the 5% level in the Duncan's Multiple Range Test for eggs laid and eggs hatched (Table V).

TABLE V

THE PERMANENCY OF STERILITY INDUCED BY TRIETHYL PHOSPHATE (TEP) AND DIETHYL CYANOMETHYLPHOSPHONATE (DECMP)

TREATMENT	TREATED DISCS*			UNTREATED DISCS*		
	EGGS LAID	EGGS HATCHED	% HATCH	EGGS LAID	EGGS HATCHED	% HATCH
Control	30.9 a	29.3	95.1 a	25.0 a	24.3	97.0 a
TEP 1.0%	24.1 b	22.0	87.9 ab	20.3 ab	19.3	95.6 a
TEP 2.0%	7.5 d	4.4	49.9 d	17.1 b	15.6	89.8 ab
DECMP 1.0%	12.7 c	8.8	73.4 bc	16.3 b	15.1	92.9 ab
DECMP 2.0%	4.4 d	3.0	67.2 c	6.1 c	5.3	87.3 b

* - Means of 10 replicates except in some untreated discs where data was missing due to death of the mites.

On removal from the treated discs, eggs laid and the percentage of eggs hatching increased in the following 48 hour period. Percentage hatch increased considerably more than eggs laid between the two intervals, with only diethyl cyanomethylphosphonate at the 2.0% concentration showing significantly reduced egg hatch at the 5% level.

The discrepancy in some treatments between the numbers of eggs laid and the percentage hatch is due to the complete cessation of egg production on some discs. Where no eggs are laid, percentage hatch cannot be calculated, therefore several discs have been eliminated from the analysis. The analysis of variance was therefore based on the missing plot technique (Finney, 1964), the sum of the squares being obtained by adjusting the degrees of freedom to the number of plots analysed (see Appendix 6).

Initial sterility tests indicated the sterilizing activity of Diethyl cyanomethylphosphonate to be greater than Triethyl phosphate (Table IV). This difference in biological activity is confirmed in the tests on the permanency of sterility (Table V), and can be related back to the differences in chemical activity. The large increase in percentage hatch on removal of the mites from the treated discs suggests the presence of two independent sites of action within the female.

RESIDUAL EFFECTS

Triethyl phosphate and diethyl cyanomethylphosphonate were tested for residual sterility effects. Adult female mites were added at intervals to leaf discs prepared and treated as described in Chapter 3. All discs were treated at the same time and the mites were added at 0, 24, 72, and 120 hours after treatment. One concentration and an untreated check were run for each period, the experiment being of a 4 x 3 factorial design. The adult mites were removed after 48 hours on the treated discs and the number of eggs laid and eggs hatched were recorded.

The results show that diethyl cyanomethylphosphonate has greater residual properties than Triethyl phosphate (Table VI). Triethyl phosphate lost all sterilizing properties after 24 hours while diethyl cyanomethylphosphonate maintained some degree of activity throughout the test period. Diethyl cyanomethylphosphonate significantly reduced egg lay in all four treatment periods (see appendix for Duncan's Multiple Range Test), and egg hatch was significantly reduced in tests at 0 and 24 hours after treatment.

The results from the experiments on the residual effects and the permanency of sterility induced by the chemosterilants suggest that diethyl cyanomethylphosphonate would be more effective for use in population control than triethyl phosphate. Further development of the former chemical will depend on investigations into the toxicology of the chemical, its possible effects on non-target organisms and the degree of phytotoxicity

TABLE VI

RESIDUAL EFFECTS OF TRIETHYL PHOSPHATE (TEP) AND DIETHYL CYANOMETHYL PHOSPHONATE (DECMP) ON THE INDUCTION OF STERILITY IN FEMALES OF *T. URTICAE*

TREATMENT	EFFECT	TIME POST-TREATMENT WHEN MITES ADDED*			
		0 Hours	24 Hours	72 Hours	120 Hours
Control	Eggs laid	25.8	23.4	25.1	23.8
	Eggs hatched	24.3	21.2	23.9	23.3
	% Hatch	95.9	91.3	95.4	97.4
TEP 2.0%	Eggs laid	10.0	21.8	22.9	18.8
	Eggs hatched	6.5	20.5	22.1	17.9
	% Hatch	54.9	94.0	96.4	95.4
DECMP 2.0%	Eggs laid	4.9	9.4	12.7	18.1
	Eggs hatched	3.1	6.5	12.2	17.1
	% Hatch	44.1	59.4	94.2	94.6

* All figures means of 10 replicates.

(See Appendix 7 for Duncan's Multiple Range Test)

CHAPTER V

GENERAL DISCUSSION

The generally held belief that sterilization of the males is more effective than female sterilization for population control, is not valid in the case of T. urticae because of its arrhenotokous type of reproduction. For this reason, and because the young adult females are easy to handle and have a short generation period, all the screening tests carried out in this research were confined to this sex and age group. Future tests of some of the active trialkyl phosphates could be extended with tests on immature stages and the males of T. urticae.

The discovery of a chemical which is suitable for use in the control of wild populations is, of course, the ultimate goal of chemosterilant screening tests. While no effort was made to suppress mite populations during this series of experiments, the ability of the chemicals tested to reduce both egg lay and egg hatch, as well as their relatively non-poisonous nature, suggests that these substances might be ideal for field use.

Chemical damage to plant tissues and the toxicity of the chemicals to animal life are two factors which may however

limit the widespread use of chemosterilants. Phytotoxicity was used as an important criterion in the rejection of chemicals from further testing. In the preliminary tests, Cycloheximide showed sterilizing activity but, at the concentration used, the chemical was severely phytotoxic. Harries (1963, 1965) and Jeppson et al. (1966) completed detailed experiments on the effects of Cycloheximide on T. urticae, but in the present investigation, the non-phytotoxic effect of some of the chemicals showing sterilizing activity resulted in the elimination of phytotoxic chemicals from further testing. Several of the trialkyl phosphates and related chemicals were also phytotoxic in the preliminary tests and were rejected from further testing. The phytotoxic response is not however, related to the sterilizing properties, but the phytotoxic effects on bean leaves were often associated with fungal growth, making it extremely difficult to follow egg development. Chemicals which show phytotoxic effects yet possess strong sterilizing properties, should not however, be completely disregarded in any further development of the chemosterilants since in most situations, T. urticae occurs on plants other than the French dwarf bean. Testing could therefore be extended onto other plant material.

Little is known of the toxicity of the trialkyl phosphates to animal life, but it is apparent that toxicities of

these compounds are much less than for most other chemosterilants. The oral LD_{50} of the aziridine, apholate, for rats is 98 mg/Kg, and the oral LD_{50} of tepa for rats is 37 mg/Kg (Gaines and Kimbrough, 1964). By comparison, 5 x 500 mg/Kg doses of trimethyl phosphate to rats was found to be just tolerable (Jackson and Jones, 1968) and recent work has shown that subtoxic concentrations (doses equivalent to 2500 to 5000 mg/Kg) administered orally or peritoneally to male mice, produced mutagenic effects (Epstein et al., 1970).

The Tetranychidae are specialised plant feeders. The feeding mechanism is by piercing the epidermis of the host by a long flagelliform stylet which is held within cheliceral modifications (Evans et al., 1961). Spider mites are thus able to pick up chemicals from within the leaf cells. In the present work, most of the trialkyl phosphates used failed to show any visable residual deposits on the surface of the leaf, suggesting a significant leaf penetration.

As described in Chapter 3, mites were added only to completely dry leaf discs, thus implying that most of the chemical is taken up by the mite through the sucking out of the juices of the plant cells. Jalil and Morrison (1969a) found a correlation between feeding activity, as measured by faecal pellet production, and egg production in T. urticae, but this result was not confirmed in the present work.

Direct studies on the method of uptake of the chemicals by spider mites will therefore be required in any future development of the chemosterilants.

CHAPTER VI

CONCLUSIONS

The conclusions which can be drawn from the results of the present investigations are as follows:

1. Preliminary screening tests have shown that chemicals of diverse structure and activity can cause partial sterility of T. urticae.

2. Trimethyl phosphate and triethyl phosphate, which are chemicals within the trialkyl phosphate group, reduced egg lay and egg hatch, while trimethyl thiophosphate reduced egg lay alone. Only the triesters containing methyl or ethyl side chains were active sterilants but ~~tri-n-butyl~~ tri-isobutyl phosphate was an active repellent and ~~tri-isobutyl~~ tri-n-butyl phosphate showed acaricidal activity.

3. Dimethyl methylphosphonate and diethyl cyanomethylphosphonate, chemical relatives of the trialkyl phosphates, reduced egg lay and egg hatch.

4. Experiments with trimethyl phosphate, triethyl phosphate, trimethyl thiophosphate, dimethyl methylphosphonate and diethyl cyanomethylphosphonate have suggested a correlation between

chemical structure and biological activity. This line of reasoning could be investigated further using a larger number of chemicals.

5. Triethyl phosphate, which is a slightly less active sterilant than trimethyl phosphate, was compared with diethyl cyanomethylphosphonate for permanency of sterility and residual activity. Diethyl cyanomethylphosphonate gave more permanent effects and had greater residual life than triethyl phosphate, suggesting that diethyl cyanomethylphosphonate is better than triethyl phosphate as a chemosterilant. Likewise, diethyl cyanomethylphosphonate showed a greater overall reduction in reproduction in spider mites than either trimethyl phosphate or triethyl phosphate.

6. The relatively low toxicity of the trialkyl phosphates and their chemical relatives suggest that these chemicals should be further investigated as potential chemosterilants for insect control. Tests could be extended to include the effects of the trialkyl phosphates on the immature stages and males of T. urticae, the effects on other insects such as the house fly, investigations into the possible systemic effects of the chemicals and the degree of population suppression occurring by application of these chemicals to a field population.

SUMMARY

The results of a search for chemicals affecting the reproduction of the two-spotted spider mite, Tetranychus urticae Koch are recorded. The arrhenotokous type of reproduction of the spider mites has meant that male sterilization does not hold any great advantage over female sterilization. The present investigation was therefore concentrated on chemicals suppressing reproduction in the females. Contiguous to this study is a review of the literature with particular reference to the range of chemicals which can act as chemosterilants, the processes of chemical induction of sterility, and the biology and habits of T. urticae. An understanding of the latter is of fundamental importance before any application of chemosterilants can be contemplated.

Preliminary tests with candidate chemosterilants were carried out and of those chemicals showing activity, the trialkyl phosphate group was selected for further study owing to the relatively low toxicity, ease of synthesis and sterilizing properties of some chemicals in this group.

Trimethyl phosphate, triethyl phosphate, dimethyl methylphosphonate and diethyl cyanomethylphosphonate reduced egg lay and egg hatch while trimethyl thiophosphate reduced egg lay alone. Experiments indicated that trimethyl phosphate was a more active reproduction suppressant than triethyl phosphate

but showed a degree of chemical instability. Triethyl phosphate was therefore used for comparison with diethyl cyanomethylphosphonate for residual effects and the permanency of sterility induced by the chemicals. Diethyl cyanomethylphosphonate had greater residual properties and the sterility was more permanent suggesting that diethyl cyanomethylphosphonate would have greater effects than triethyl phosphate on the mite populations if these chemicals were used for population suppression.

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APPENDICES

Appendix 1: Antifertility Effects of Trimethyl Phosphate.

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	26	26	28	25	30	34	27	25	25	27	27.3
	Eggs hatched	24	25	28	23	27	33	26	25	25	23	25.9
	% hatch*	92	96	100	94	93	98	96	100	100	85	95.4
2.5%	Eggs laid	17	9	7	13	7	21	14	9	5	6	10.8
	Eggs hatched	16	0	1	0	0	10	4	1	0	1	3.3
	% hatch*	94	0	14	0	0	48	29	11	0	17	21.3
1.5%	Eggs laid	26	20	20	16	28	26	16	10	15	14	19.1
	Eggs hatched	21	16	15	6	26	24	6	4	13	7	13.8
	% hatch*	81	80	75	37	93	92	37	40	87	50	67.2

* taken to nearest whole number

Duncan's Test: (P=<0.01) (P=<0.05)

1) EGGS LAID

	$\bar{x}$	1%	5%
Control	27.3	A	a
1.5%	19.1	B	b
2.5%	10.8	C	c

SE mean 1.15 (Standard Error of the Mean)

2) PERCENTAGE HATCH

Treatment	$\bar{x}$	1%	5%
Control	95.4	A	a
1.5%	67.2	B	b
2.5%	21.3	C	c

SE Mean 6.99

Appendix 1 continued.

Analysis of Variance:

1) EGGS LAID

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	1367.27	683.63	22.11**
Error	27	834.60	30.91	
Total	29	2201.87		

SE = 5.56 (Standard Error)

C.V. = 29.68 (Coefficient of Variation)

2) PERCENTAGE HATCH

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	27976.20	13988.10	28.62**
Error	27	13196.10	488.74	
Total	29	41172.30		

SE = 22.11

C.V. = 36.06

Appendix 2: Antifertility Effects of Triethyl Phosphate.

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	23	21	22	22	23	25	21	25	17	21	22.0
	Eggs hatched	22	20	22	22	22	25	21	22	16	21	21.3
	% hatch*	96	95	100	100	96	100	100	88	94	100	96.9
2.5%	Eggs laid	9	3	8	7	9	7	8	10	9	10	8.0
	Eggs hatched	6	2	5	6	7	2	5	6	7	7	5.3
	% hatch*	67	67	63	86	78	29	63	60	78	70	66.1
1.5%	Eggs laid	20	19	14	18	11	15	10	9	12	13	14.1
	Eggs hatched	16	19	13	18	10	14	9	9	12	12	13.2
	% hatch*	80	100	93	100	91	93	90	100	100	92	93.9

* taken to nearest whole number

Duncan's Test: (P=<0.01) (P=<0.05)

1) EGGS LAID

	$\bar{x}$	1%	5%
Control	22.0	A	a
1.5%	14.1	B	b
2.5%	8.0	C	c

SE Mean 0.90

2) PERCENTAGE HATCH

Treatment	$\bar{x}$	1%	5%
Control	96.9	A	a
1.5%	93.9	A	a
2.5%	66.1	B	b

SE Mean 3.13

Appendix 2 continued.

Analysis of Variance:

1) EGGS LAID

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	985.40	492.70	60.77**
Error	27	218.90	8.11	
Total	29	1204.30		

SE = 2.85 C.V. = 19.37

2) PERCENTAGE HATCH

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	5768.27	2884.13	29.48**
Error	27	2640.70	97.80	
Total	29	8408.97		

SE = 9.89 C.V. = 11.55

Appendix 3: Antifertility Effects of Trimethyl Thiophosphate.

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	25	23	21	22	19	19	20	21	25	19	21.4
	Eggs hatched	23	22	21	22	19	18	19	21	25	17	20.7
	% hatch*	92	96	100	100	100	95	95	100	100	90	96.8
0.05%	Eggs laid	12	8	2	3d	5	7	7	1d	8	8	6.1
	Eggs hatched	12	6	2	3	5	7	7	1	7	6	5.7
	% hatch*	100	75	100	100	100	100	100	100	88	75	96.3
0.025%	Eggs laid	15	14	17	15	16	5	10d	23	14	8	13.7
	Eggs hatched	15	14	16	12	16	5	10	22	11	8	12.9
	% hatch*	100	100	94	55	100	100	100	96	48	100	88.7

* taken to nearest whole number

Duncan's Test: ($P=<0.01$) ($P=<0.05$)

1) EGGS LAID

	$\bar{x}$	1%	5%
Control	21.4	A	a
0.05%	6.1	B	b
0.025%	13.7	C	c

SE Mean 1.18

2) PERCENTAGE HATCH

Treatment	$\bar{x}$	1%	5%
Control	96.8	A	a
0.05%	96.3	A	a
0.025%	88.7	A	a

SE Mean 3.99

Appendix 3 continued.

Analysis of Variance:

1) EGGS LAID

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	1170.47	585.23	41.86**
Error	27	377.40	13.98	
Total	29	1547.87		

SE = 3.74 C.V. = 27.22

2) PERCENTAGE HATCH

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	412.07	206.03	1.29
Error	27	4299.80	159.25	
Total	29	4711.87		

SE = 12.62 C.V. = 13.43

Appendix 4: Antifertility Effects of Dimethyl Methylphosphonate.

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	29	21	29	28	26	28	14	28	26	28	25.7
	Eggs hatched	29	21	19	28	25	28	13	27	23	28	24.1
	% hatch*	100	100	66	100	96	100	93	97	88	100	94.0
7.5%	Eggs laid	5	0	3	18	2	10	14	0	1	13	6.6
	Eggs hatched	5	0	3	16	2	1	8	0	0	1	3.6
	% hatch*	100	-	100	89	100	10	57	-	0	8	66.3
2.5%	Eggs laid	19	8	16	18	28	26	11	26	23	21	19.6
	Eggs hatched	10	1	8	8	19	12	1	19	11	14	10.3
	% hatch*	53	12	50	23	68	46	9	73	48	67	44.9
1.5%	Eggs laid	11	8	8	29	4	27	30	21	30	15	18.3
	Eggs hatched	8	2	3	17	3	24	20	18	22	4	12.1
	% hatch*	73	25	38	59	75	89	67	86	73	19	60.4

* taken to nearest whole number

Duncan's Test: (P=<0.01) (P=<0.05)

1) EGGS LAID

	$\bar{x}$	1%	5%
Control	25.7	A	a
2.5%	19.6	A B	a
1.5%	18.3	B	a
7.5%	6.6	C	b

SE Mean 2.32

2) PERCENTAGE HATCH*

Treatment	$\bar{x}$	1%	5%
Control	94.0	A	a
1.5%	60.4	B	b
2.5%	44.9	B	b

SE Mean 6.47

* Treatment 7.5% was not analysed due to the wide variation in percentage hatch.

Appendix 4 continued.

Analysis of Variance:

1) EGGS LAID

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	3	1910.90	636.97	11.83**
Error	36	1937.00	53.81	
Total	39	3847.90		

SE = 7.34 C.V. = 41.77

2) PERCENTAGE HATCH

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	2	12600.07	6300.03	15.05**
Error	27	11297.30	418.42	
Total	29	23897.37		

SE = 20.46 C.V. = 30.79

Appendix 5: Antifertility Effects of Triethyl Phosphate (TEP) and Diethyl Cyanomethylphosphonate (DECMP)

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	29	10	32	27	33	26	27	30	31	28	27.3
	Eggs hatched	27	10	31	27	33	26	26	29	31	28	26.8
	% hatch*	94	100	97	100	100	100	96	97	100	100	98.4
DECMP 2.0%	Eggs laid	5	3	0	3	5	3	6	5	9	0	3.9
	Eggs hatched	0	3	0	1	2	1	5	2	4	0	1.8
	% hatch*	0	100	-	33	40	33	83	40	44	-	46.6
TEP 2.0%	Eggs laid	9	4	8	5	10	9	6	11	8	2	7.2
	Eggs hatched	7	2	4	3	6	7	5	10	7	2	5.3
	% hatch*	78	50	50	60	60	78	83	91	88	100	73.8
TEP 1.0%	Eggs laid	0	17	13	3	4	0	14	12	6	9	7.8
	Eggs hatched	0	17	10	3	2	0	10	10	5	8	6.5
	% hatch*	-	100	77	100	50	-	71	83	83	89	81.6
DECMP 1.0%	Eggs laid	17	20	1	20	13	9	13	0	24	14	13.1
	Eggs hatched	15	19	0	19	10	8	11	0	22	14	11.8
	% hatch*	88	95	0	95	77	89	85	-	92	100	80.1

* taken to nearest whole number

Duncan's Test:

1) EGGS LAID (P=<0.01) (P=<0.05)

Treatment	$\bar{x}$	1%	5%
Control	27.3	A	a
DECMP 1.0%	13.1	B	b c
TEP 1.0%	7.8	C D	c d
TEP 2.0%	7.2	D	c d
DECMP 2.0%	3.9	D	d

SE Mean 1.78

Appendix 5 continued.

2) PERCENTAGE HATCH

Treatment			
Control	98.4	A	a
TEP 1.0%	81.6	A	a b
DECMP 1.0%	80.0	A	a b
TEP 2.0%	73.8	A	b
DECMP 2.0%	46.6	B	c
SE Mean	6.90		

Analysis of Variance:

1) EGGS LAID

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	4	3414.92	853.73	27.11**
Error	45	1417.10	31.49	
Total	49	4832.02		

SE = 5.61 C.V. = 47.32

2) PERCENTAGE HATCH

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	4	12321.00	3080.30	6.45**
Error	40	19114.70	477.90	
Total	44	31435.70		

SE = 21.9 C.V. = 28.4

Appendix 6: The Permanency of Sterility Induced by Triethyl Phosphate (TEP) and Diethyl Cyanomethylphosphonate (DECMP)

A) TREATED DISCS

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	30	30	32	30	31	28	35	33	30	30	30.9
	Eggs hatched	28	27	31	30	28	28	30	33	28	30	29.3
	% hatch*	93	90	97	100	90	100	86	100	93	100	94.9
DECMP 2.0%	Eggs laid	6	0	15	0	8	6	0	0	6	3	4.4
	Eggs hatched	3	0	11	0	5	5	0	0	4	2	3.0
	% hatch*	50	-	73	-	63	83	-	-	67	67	67.2
TEP 2.0%	Eggs laid	12	7	15	0	4	2	8	12	6	9	7.5
	Eggs hatched	9	2	12	0	2	1	3	6	3	6	4.4
	% hatch*	75	29	40	-	50	50	38	50	50	67	49.9
DECMP 1.0%	Eggs laid	17	15	0	13	16	1	5	25	21	14	12.7
	Eggs hatched	8	10	0	12	1	1	5	24	17	10	8.8
	% hatch*	47	67	-	92	6	100	100	96	81	72	73.4
TEP 1.0%	Eggs laid	17	21	30	27	30	32	13	25	26	20	24.1
	Eggs hatched	14	17	29	24	29	31	7	25	25	19	22.0
	% hatch*	82	81	97	80	97	97	54	100	96	95	87.9

* taken to nearest whole number

Duncan's Test:

1) EGGS LAID - Treated discs ($P=<0.01$) ($P=<0.05$)

Treatment	$\bar{x}$	1%	5%
Control	30.9	A	a
TEP 1.0%	24.1	B	b
DECMP 1.0%	12.7	C	c
TEP 2.0%	7.5	C D	d
DECMP 2.0%	4.4	D	d
SE Mean	1.77		

Appendix 6 continued.

2) PERCENTAGE HATCH - Treated discs

Treatment	$\bar{x}$	1%	5%
Control	95.1	A	a
TEP 1.0%	87.9	A B	a b
DECMP 1.0%	73.4	A B	b c
DECMP 2.0%	67.2	B	c
TEP 2.0%	49.9	C	d
SE Mean	5.5		

Analysis of Variance:

1) EGGS LAID

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	4	5052.88	1263.22	40.40**
Error	45	1406.80	31.26	
Total	49	6459.68		

SE = 5.59 C.V. = 35.12

2) PERCENTAGE HATCH

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	4	11727.3	2931.8	9.67**
Error	39	11823.7	303.2	
Total	43	23551.0		

SE = 17.4 C.V. = 22.9

Appendix 6 continued.

B) UNTREATED DISCS

Treatment	Effect	Replicates										
		1	2	3	4	5	6	7	8	9	10	$\bar{x}$
CONTROL	Eggs laid	26	29	25	23	D	26	D	27	19	D	25.0
	Eggs hatched	25	29	24	21	D	25	D	27	19	D	24.3
	% hatch*	96	100	96	91	-	96	-	100	100	-	97.0
DECMP 2.0%	Eggs laid	D	4	20	16	1	0	D	1	0	5	6.1
	Eggs hatched	D	3	19	15	1	0	D	1	0	3	5.3
	% hatch*	-	75	95	94	100	-	-	100	-	60	87.3
TEP 2.0%	Eggs laid	18	17	28	23	D	22	4	8	14	20	17.1
	Eggs hatched	18	16	25	20	D	21	3	8	11	18	15.6
	% hatch*	100	94	89	87	-	95	75	100	96	100	89.8
DECMP 1.0%	Eggs laid	22	21	0	23	24	19	0	21	17	16	16.3
	Eggs hatched	22	21	0	23	17	18	0	20	15	15	15.1
	% hatch*	100	100	-	100	71	95	-	95	88	94	92.9
TEP 1.0%	Eggs laid	9	8	26	D	32	10	26	29	22	21	20.3
	Eggs hatched	8	8	25	D	29	10	23	29	21	21	19.3
	% hatch*	89	100	96	-	91	100	88	100	96	100	95.6

* taken to nearest whole number

D - Mite dead when transferred from treated disc.

Duncan's Test:

1) EGGS LAID - Untreated discs (P=<0.01) (P=<0.05)

Treatment	$\bar{x}$	1%	5%
Control	25.0	A	a
TEP 1.0%	20.3	A	a b
TEP 2.0%	17.1	A	b
DECMP 1.0%	16.3	A	b
DECMP 2.0%	6.1	B	c
SE Mean	2.6		

Appendix 6 continued.

2) PERCENTAGE HATCH - Untreated discs

Treatment	$\bar{x}$	1%	5%
Control	97.0	A	a
TEP 1.0%	95.6	A	a
DECMP 1.0%	92.9	A	a b
TEP 2.0%	89.8	A	a b
DECMP 2.0%	87.3	A	b
SE Mean	2.9		

Analysis of Variance:

Due to	df	Sums of Sq's	Mean Sq	F-test
Treatments	4	1541.2	385.3	6.33**
Error	38	2321.9	61.1	
Total	42	3863.1		

SE = 7.8 C.V. = 46.4

Appendix 7: Residual Effects of Triethyl Phosphate (TEP) and Diethyl Cyanomethylphosphonate (DECMP) on the Induction of Sterility in Females of T. urticae

Treatment		Effect	Replicates										
			1	2	3	4	5	6	7	8	9	10	$\bar{x}$
0 HOURS	Control	Eggs laid	23	26	28	26	23	26	27	24	24	26	25.3
		% hatch	87	100	100	92	96	92	92	100	100	100	95.9
	TEP 2.0%	Eggs laid	9	2	0	14	6	11	8	7	24	19	10.0
		% hatch	56	50	-	71	50	82	63	43	71	63	54.9
	DECMP 2.0%	Eggs laid	8	5	4	0	0	5	1	10	12	4	4.9
		% hatch	38	40	100	-	-	60	0	70	83	50	44.1
24 HOURS	Control	Eggs laid	28	24	24	18	20	25	24	25	22	24	23.4
		% hatch	79	96	96	95	95	100	100	52	100	100	91.3
	TEP 2.0%	Eggs laid	23	20	24	19	23	22	19	27	22	19	21.8
		% hatch	100	90	92	100	91	91	100	96	91	89	94.0
	DECMP 2.0%	Eggs laid	0	13	7	20	7	4	2	13	24	4	9.4
		% hatch	-	54	14	70	29	75	100	85	92	75	59.4

(Continued)

Appendix 7 continued.

Treatment		Effect	Replicates										
			1	2	3	4	5	6	7	8	9	10	$\bar{x}$
72 HOURS	Control	Eggs laid	22	22	21	31	21	17	28	24	26	29	25.1
		% hatch	100	100	91	77	100	94	100	95	97	100	95.4
	TEP 2.0%	Eggs laid	17	23	24	30	21	17	28	24	26	19	22.9
		% hatch	100	100	96	97	95	100	96	100	96	84	96.4
	DECMP 2.0%	Eggs laid	7	14	2	23	4	15	6	13	26	12	12.2
		% hatch	100	100	67	96	100	100	100	87	100	92	94.2
120 HOURS	Control	Eggs laid	18	21	22	23	24	29	29	20	31	21	23.8
		% hatch	100	95	100	95	96	97	97	100	94	100	97.4
	TEP 2.0%	Eggs laid	17	22	14	14	16	27	19	22	22	15	18.8
		% hatch	100	95	100	79	100	93	95	96	96	100	95.4
	DECMP 2.0%	Eggs laid	28	16	20	14	13	14	18	20	18	20	18.1
		% hatch	93	94	90	100	100	86	100	100	83	100	94.6

Appendix 7 continued.

Duncan's Test:

1) EGGS LAID

Treatment	Hours	$\bar{x}$	(P=<0.01) 1%	(P=<0.05) 5%
Control	0	25.3	A	a
Control	72	25.1	A B	a
Control	120	23.8	A B	a b
Control	24	23.4	A B	a b
TEP	72	22.9	A B	a b c
TEP	24	21.8	A B	a b c
TEP	120	18.8	A B C	b c
DECMP	120	18.1	B C	c
DECMP	72	12.7	C D	d
TEP	0	10.0	D E	d
DECMP	24	9.4	D E	d e
DECMP	0	4.9	E	e

SE Mean 1.64

2) PERCENTAGE HATCH

Treatment	Hours	$\bar{x}$	1%	5%
Control	120	97.4	A	a
TEP	72	96.4	A	a
Control	0	95.9	A	a
Control	72	95.4	A	a
TEP	120	95.4	A	a
DECMP	120	94.6	A	a
DECMP	72	94.2	A	a
TEP	24	94.0	A	a
Control	24	91.3	A	a
DECMP	24	59.4	B	b
TEP	0	54.9	B	b
DECMP	0	44.1	B	b

SE Mean 5.40

Appendix 7 continued.

Analysis of Variance:

1) EGGS LAID

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	11	5432.57	493.87	18.25**
Hours	3	935.23	311.74	11.52**
Chemicals	2	3453.02	1726.51	63.32**
Hours x Chemicals	6	1044.32	174.05	6.43**
Error	108	2921.40	27.05	
Total	119	8353.97		

SE = 5.20 C.V. = 28.87

2) PERCENTAGE HATCH

Due to	df	Sum of Sq's	Mean Sq	F-test
Treatments	11	41464.36	3769.49	12.91**
Hours	3	19055.37	6351.79	21.76**
Chemicals	2	9648.62	4824.31	16.53**
Hours x Chemicals	6	12760.38	2126.73	7.28**
Error	108	31514.80	291.80	
Total	119	72979.16		

SE = 17.08 C.V. = 20.24