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THURINGIENSIN TOXICITY TO *Tetranychus urticae* KOCH
AND *Panonychus ulmi* (Koch) (TETRANYCHIDAE)
AND EFFECTS ON CUTICLE DEVELOPMENT
OF IMMATURE STAGES OF *T. urticae*

A thesis submitted in partial fulfilment
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by
R.R. Vargas M.

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THURINGIENSIN TOXICITY TO Tetranychus urticae KOCH
AND Panonychus ulmi (KOCH) (TETRANYCHIDAE)
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By R.R. Vargas M.

Thuringiensin is a novel pesticide that has been evaluated against a range of insects and spider mites. It is generally more effective against immature stages than adults of spider mites, but there is considerable variation in the published data on this active ingredient. Current reviews have suggested that differences in bioassay methods, formulations, and species may be responsible for these variations. Therefore a more systematic and intensive investigation was carried out, where the mode of action of thuringiensin on cuticle synthesis inhibition was specially taken into account.

The direct and residual toxicity of thuringiensin to different stages of T. urticae and P. ulmi and the influence on T. urticae reproduction and population development were evaluated in laboratory bioassays. Direct toxicity was higher than residual toxicity to T. urticae for all life stages. Immature stages of T. urticae and P. ulmi were more susceptible
to thuringiensin than adults. *P. ulmi* was more susceptible to thuringiensin than *T. urticae*. Fecundity of *T. urticae* was significantly reduced when females were exposed to residues for 2 d. Complete suppression of *T. urticae* population development was achieved when the F1 generation was exposed to thuringiensin residues; however, the level of suppression was concentration dependent.

The effects of temperature, host plant, active ingredient mobility, surfactant, residue age and larval age on the response *T. urticae* to thuringiensin were also evaluated in laboratory bioassays. *T. urticae* larvae were significantly more susceptible to thuringiensin at 13°C than those at 28°C. A significantly different response of *T. urticae* larvae to thuringiensin residues on peach and apple leaves was found. Older *T. urticae* larvae were significantly more susceptible than younger larvae when exposed to thuringiensin residues. No systemic or translaminar effects of thuringiensin were detected; leaf side and surfactant did not affect the efficacy of thuringiensin.

The effect of thuringiensin on the ultrastructure of *T. urticae* cuticle was investigated using an electron microscope. Thuringiensin was found to affect epicuticle synthesis in larvae, protonymphs and deutonymphs. The disruptive effects of thuringiensin on larval cuticle formation was observed from 12 h onwards, indicating that the effect of the time of exposure of mites to thuringiensin is related to the stage of cuticle development. The results suggest that the effects of thuringiensin occur in a relatively short time.
Therefore the high toxicity of thuringiensin to immature spider mites stages and the sublethal effects on females suggest that thuringiensin may successfully control field populations and the effect of host plant and temperature may enhance the effectiveness of thuringiensin. The use of thuringiensin in spider mites control programmes is discussed. Practical suggestions on the development of bioassays for active ingredients of this type are also discussed.

Keywords: Thuringiensin, β-exotoxin, miticide, direct toxicity, residual toxicity, *Tetranychus urticae*, *Panonychus ulmi*, bioassay, temperature, residue age, host plant, surfactant, translocation, cuticle development, immature stages, ultrastructure.
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Chapter 1

Introduction

PHYTOPHAGOUS SPIDER MITES (Acari:Tetranychidae) are present throughout the temperate and tropical regions of the world and are frequently major pests of food, fibre and ornamental crops (Helle & Sabelis 1985). Two spider mite species, *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch) occur on many agricultural and horticultural crops (van de Vrie et al. 1972) and are the key spider mite pest of numerous crops in New Zealand (Penman 1984). Although integrated mite control (IMC) programmes involving predatory phytoseid mites have been established in a range of crops (Schroder 1982, Helle & Sabelis 1985), application of miticides still remains an important method for controlling spider mites in New Zealand (Chapman & Penman 1987) and other countries (Dennehy et al. 1990). Chemical control of both species is achieved through a narrow range of specific miticides. However, in recent years resistance, intolerance of residues on export produce, and toxicological problems have barred the use of several
conventional miticides, e.g., cyhexatin.

New Zealand exports each year a variety of fresh fruits, vegetables and flowers which must meet the phytosanitary requirements of importing countries (Bollard & Weston 1983). To keep export crops free of pests intensive applications of pesticides are made, however, such use can lead to the development of resistance (Elliot et al. 1988). Spider mites have a remarkable ability to develop pesticide resistance due to their high fecundity, multivoltinism, mode of reproduction, and limited within-plant dispersal (Hussey & Parr 1963). *T. urticae* and *P. ulmi* have accumulated a considerable number of genes conferring resistance to all major classes of miticides and, in some instances, nearly all of the previously effective miticides have been depleted (Georghiou 1990).

When resistance develops, alternative miticides or control methods are required to suppress populations below damage threshold levels. Unfortunately, the supply of new miticides from different chemical groups is limited and also they are not readily available to replace redundant products, since the development and registration of new products has slowed markedly (Buchanan 1991).

Research into alternatives for conventional pesticides has followed many directions. One that showed considerable promise has been the exotoxins produced by *Bacillus thuringiensis* Berliner. A β-exotoxin, thuringiensin, produced by strains of several serotype groups of *B. thuringiensis* during vegetative growth, has attracted considerable attention. This toxin, an analogue of ATP, inhibits RNA polymerase and subsequently cell
replication (mitosis) by competing with ATP for enzymatic binding sites (Sebesta et al. 1981). This subcellular process occurs more slowly than the effects caused by nerve poisons and mortality is frequently not apparent until critical growth stages such as moulting or pupation occur (Forrester 1993).

Thuringiensin is active on a wider spectrum of pests than *B. thuringiensis* including mites and some insects belonging to the Orders Coleoptera, Diptera, Hemiptera and Lepidoptera (Sebesta et al. 1981).

Krieg (1968) was the first to prove the effectiveness of a supernatant of a β-exotoxin on active stages of *T. urticae*. The following studies have shown that thuringiensin has been more effective against immature stages than adults of *P. citri* (Hall et al. 1971), *T. urticae* and *T. pacificus* (Grau 1986), *T. pacificus* (Perring & Farrar 1986, Hoy & Ouyang 1987) and *T. urticae* (Royalty et al. 1990) and *P. ulmi* (Royalty et al. 1991). Thuringiensin has also been shown to be toxic to adult female *Metaseiulus occidentalis* (Nesbitt) (Hoy & Ouyang 1987), a predatory phytoseiid mite species commonly employed in IMC programmes.

Although thuringiensin has been tested against spider mites by exposing them to residues and direct spray contact, no comparative studies have been carried out to resolve the question about the most effective exposure route (Neal et al. 1987; Royalty et al. 1990, 1991). There is also uncertainty about the speed at which thuringiensin acts. Some researchers (Krieg 1968, Royalty et al. 1990, 1991) have reported that thuringiensin acts very slowly while Neal et al. (1987) found
that it was highly effective against motile forms within 2 d of exposure. There is also some doubt about the relative effectiveness of thuringiensin against different mite life cycle stages. Some tests have indicated effective control of immature mite stages (Royalty et al. 1990, 1991) while other studies have shown high toxicity to both immatures and adults (Neal et al. 1987). These variable results may be due to different bioassay methods, formulations, species, strains, etc., and therefore demonstrate a need to investigate the toxicity of thuringiensin in a more systematic and intensive manner. The development and use of an appropriate bioassay method, where the mode of action of thuringiensin is taken into account, will be essential to determine reliable estimates of toxicity.

Factors that might influence the toxicity of thuringiensin to spider mites have not yet been reported. An understanding of these factors is important because several environmental and biological factors such as temperature (Everson & Tonks 1981; James et al. 1988), host plant (Wakou & Sugawara 1974, Asano & Kamei 1982, Marris & Chapman 1987), mobility through the plant or over the surface (Munthali & Wyatt 1986), residue persistence (Hall et al. 1971, Neal et al. 1987), and the age of mites (Aveyard et al. 1986; Welty et al. 1988; Marshall & Pree 1991) have been shown to influence the responses of spider mites to other miticides. Therefore it will be important to determine what factors may influence thuringiensin toxicity in order to maximise its effectiveness.

Thuringiensin has been reported to be an efficient inhibitor of cuticle synthesis in insects and mites (Sebesta et
al. 1981) and is similar in many respects to the effects exerted by other cuticle synthesis inhibitors like aminopterin, methotrexate, cyromazine, puromycin, cyclohexamine and several benzoylphenyl ureas (Binnington & Retnakaran 1991). Although the external manifestation of moult disruption is similar to that observed with other cuticle inhibitors where juvenile individuals are unable to reach the succeeding chrysalid stage, the biochemical processes involved and the structures disrupted differ between inhibitors (Oberlander et al. 1980, Chen & Riddiford, 1981; Sebesta et al. 1981, Mothes-Wagner & Seitz 1982, Retnakaran et al. 1985). Ultrastructural studies have been identified as an important method to investigate the mechanism of action of other inhibitors of cuticle formation (Cohen 1987b, Mothes-Wagner & Seitz 1981, Mothes-Wagner 1986) and because the effects of thuringiensin on cuticle formation in spider mites have not been investigated, such a study would be desirable.

The first aim of this study was to determine the responses of susceptible populations of *T. urticae* and *P. ulmi* to thuringiensin. Specific objectives under this aim included:

a) selecting and developing an appropriate bioassay method;

b) characterizing the responses of all immature stages and adults to thuringiensin by both direct and residue exposure routes;

c) determining the effects of thuringiensin on egg production in *T. urticae*;

d) quantifying the effects of thuringiensin on *T. urticae*
population development.

The second aim of this study was to investigate the effect of a range of factors that could influence the toxicity of thuringiensin to *T. urticae*. Specific objectives under this aim included:

a) evaluating the effects of temperature, host plant, active ingredient mobility, surfactant and residue age on the responses of *T. urticae* to thuringiensin;
b) determining the responses of three *T. urticae* larval ages to thuringiensin.

The third aim of this study was to determine the effect of thuringiensin on cuticle development in *T. urticae*. Specific objectives under this aim included:

a) developing methods to study the cuticle ultrastructure of *T. urticae* immature stages;
b) determining what cuticle structures are affected by thuringiensin;
c) determining the critical period in larval development when thuringiensin has its greatest effect.

The results of this thesis are presented in the following three chapters, each of which has been written in the form of a manuscript suitable for submission to the *Journal of Economic Entomology*. These chapters are followed by a general discussion that integrates these results and evaluates the significance and applications of this research.
Chapter 2

Toxicity of Thuringiensin to Immature Stages and Adults of *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch) (Acarina: Tetranychidae).

**Introduction**

SPIDER MITES are economically important pests on a wide range of agricultural and horticultural crops. The main pest species on horticultural crops in New Zealand are European red mite *Panonychus ulmi* (Koch) and twospotted spider mite *Tetranychus urticae* Koch. Chemical control of both species is commonly achieved with a narrow range of acaricides. However, resistance, intolerance of residues on export produce, selectivity to beneficial species, toxicological and environmental problems have severely restricted the use of several conventional acaricides. To overcome these problems, the search for alternatives to conventional pesticides has intensified over the last decade (Roush & Tabashnik 1990). The
introduction of any new acaricide therefore represents a timely addition to a depleted selection of chemicals.

Thuringiensin (β-exotoxin, Abbott Laboratories, Chicago, USA) has several characteristics that may make it suitable for use in spider mite control programmes. Thuringiensin is a water-soluble, dialyzable nucleotide composed of adenine, ribose, glucose, and allaric acid with a phosphate group (Farkas et al. 1969). Thuringiensin is secreted externally from *Bacillus thuringiensis* Berliner cells into the culture medium during vegetative growth. Heat-tolerant exotoxins (β-exotoxins) were discovered by McConnell and Richards (1959); these are known as β-exotoxin, fly factor, heat-stable toxin, thermostable toxin, and thuringiensin. Studies by Mohd-Salleh et al. (1980) and Gingrinch et al. (1992a, 1992b) indicated the existence of more than one type of heat-tolerant exotoxin. The existence of a second heat-tolerant exotoxin was confirmed chemically by Levinson et al. (1990). They called this toxin a type II β-exotoxin, which was more specific than the type I β-exotoxin, and was specially active against *Leptinotarsa decemlineata* (Say).

Type I β-exotoxin, thuringiensin, acts by inhibiting ribosomal DNA-dependent RNA polymerase and competes with ATP for enzymatic binding sites (Mohd-Salleh et al. 1980). Toxicity is expressed when high growth rates and physiological processes (e.g., metamorphosis) occur in immature insects and mites. These processes require higher rates of RNA synthesis than are necessary for the comparatively slower growth of adults (Sebesta et al. 1981). Type I β-exotoxin may have contact toxicity to
arthropods, as well as having oral toxicity (Neal et al. 1987, Grau 1986, Hoy & Ouyang 1987).

Little is known of the effects of thuringiensin on spider mites. Krieg (1968) proved the effectiveness of a supernatant of a β-exotoxin on active stages of T. urticae, whereas Hall et al. (1971) found that β-exotoxin was highly toxic to adult and immature Panonychus citri (McGregor). Grau (1986) showed that a pure preparation of β-exotoxin (A.B.G. 6162) was toxic to T. urticae and Tetranychus pacificus McGregor in cotton. However, Perring & Farrar (1986) observed no control of T. urticae on melon. Hoy & Ouyang (1987) found that β-exotoxin was toxic to adult females of T. pacificus and Metaseiulus occidentalis (Nesbitt) within 48-96 h when they were treated at rates of 0.125-4 times the proposed field rate (0.0528 g AI/litre).

More recently, Royalty et al. (1990, 1991) discovered that the toxicity of two formulations of thuringiensin to T. urticae and P. ulmi was not significantly different to protonymphs and deutonymphs. However, both these immature stages were significantly more susceptible than adults, although only low mortality (12.5%) of immature stages was recorded after 3 d. The toxicity to immature stages and the sublethal effects on adults suggests that thuringiensin may control field populations of T. urticae and P. ulmi, despite causing low initial mortality.

The specific objectives of this study were to compare the direct and residual toxicity of thuringiensin to different life stages of T. urticae and P. ulmi and to determine the effects of thuringiensin on T. urticae reproduction and population
development.

**Materials and Methods**

A series of bioassays was done to determine the direct and residual toxicity of thuringiensin to *T. urticae* and the direct toxicity to *P. ulmi*. In addition, experiments were done to determine the effects of thuringiensin on *T. urticae* fecundity, egg hatch and population development. However these tests were not repeated with *P. ulmi* because of rearing difficulties in the laboratory.

**Sources of Mites.** A *T. urticae* strain has been maintained by the Department of Entomology, Lincoln University, Canterbury since 1985 without exposure to pesticides. Mites were reared on French dwarf bean (*Phaseolus vulgaris*, cv. 'Tendergreen') in the laboratory; the photoperiod was 16:8(L:D). The temperature and humidity were not controlled but were approximately 21 ± 3°C and 60 ± 15% RH. New plants were added to the colony when required.

*P. ulmi* was collected initially from peach trees grown in the Horticultural Research Area, Lincoln University, and placed on peach (*Prunus persica*, cv. 'Red Haven') grown under laboratory and glasshouse conditions. Temperature and humidity conditions were not controlled but were similar to those for *T. urticae* rearing.

**Miticide.** An experimental formulation of thuringiensin (ABG-6320), an aqueous suspension (5% AI) supplied by Abbott
Laboratories, USA, was used in all experiments. The physical and chemical properties of thuringiensin have previously been described by Sebesta et al. (1981).

**Bioassays.** A Potter tower (Burkard Manufacturing Co. Ltd, Rickmansworth, U.K.) was used for applying thuringiensin suspensions in all experiments. Two ml of thuringiensin suspension was sprayed on each occasion at 55 ± 5 kPa; a 10 s settling period followed. This technique resulted in a wet deposit of 1.25 ± 0.01 mg/cm² (ten 22 mm dia glass microscope slide coverslips were weighed before and after spraying to obtain the mean wet deposit).

When a concentration-mortality response was to be estimated assuming the probit model, preliminary experiments with a small number of mites were done to select a series of five concentrations that would produce 5-95% mortality (Robertson et al. 1984). All mites used in these bioassays were carefully selected; these mites were approximately half-way through any developmental stage.

Because an observation period of > 24 h was necessary to evaluate the moult-inhibition effects of thuringiensin, a whole-leaf method was used in the bioassays. Because whole French dwarf bean leaves were used, mites were able to reach adulthood without depleting the food resource. The excised leaf was placed upside down on moistened cotton wool in a 85 mm diameter Petri dish. Run-off from the leaves was prevented by confining mites in 12 mm diameter arenas surrounded by a sticky insect trap adhesive (Davis Gelatine N.Z. Ltd, Christchurch). Up to 25
mites were placed into each arena. At least four replicates for each concentration were tested (one replicate per leaf). Successive experiments were done with larvae, protonymphs, deutonymphs, and adults. Controls with a similar number of mites but treated only with water were included in tests with each stage.

On experiments where leaf discs were used, five discs (12 mm diameter) were placed on moistened cotton wool in a Petri dish. Otherwise experiments using leaf discs were the same as for experiments using whole leaves.

In all experiments, mites were held at 23 ± 2°C and a photoperiod of 16:8(L:D), until the control group mites reached adulthood. Mites were considered dead when individuals did not reach the succeeding chrysalid stage during an interval equivalent to that in the control groups (approximately 2 d for each immature stage). Adults were considered dead if they were unable to move at least one body length when lightly prodded or were stuck to the leaf surface.

**Direct and Residual Toxicity.** The whole-leaf method was used to determine the direct and residual toxicity of thuringiensin to *T. urticae*, whereas the leaf disc method was used to determine the direct toxicity to *P. ulmi*. After mites were placed in the arenas, the leaves or leaf discs were sprayed in the Potter tower with a range of five thuringiensin concentrations; applications were made from lowest to highest concentration, after controls were treated with water only. To determine the residual toxicity of thuringiensin to *T. urticae*,
the mites were placed into the arenas after residues had air-dried for 15 min.

Besides determining the direct and residual toxicity of thuringiensin, the importance of oral ingestion of thuringiensin was also investigated with *T. urticae* larvae. Two types of substrates for arenas were used. These were glass Petri dishes that would prevent feeding, and French dwarf bean leaves that would allow feeding. One thuringiensin concentration was used (0.005 g AI/litre) to test the response of mites on both substrates. Groups of 25 larvae were placed in each arena after the residues had dried for 15 min. After 8 h, larvae were removed and placed on residue-free leaves. To determine the effect of direct spray only, 4 groups of 25 larvae were placed on glass Petri dishes, sprayed (0.005 g AI/litre) and transferred immediately to arenas on fresh residue-free French dwarf bean leaves. Each arena was considered as a replicate (one replicate per leaf or Petri dish).

**Fecundity.** The effect of thuringiensin on fecundity *T. urticae* was determined by exposing females to direct sprays or residues for varying periods. Twenty females 1-d-old were placed on French dwarf bean leaves immediately after the residues had dried, or they were sprayed directly and left on the residues. A sublethal concentration of thuringiensin equivalent to approximately 0.1 of the direct adult LC$_{50}$ (0.06 g AI/litre) was initially selected and used to determine the sterilising effect on females. A 12 mm diameter arena placed on the lower surface of one primary French dwarf bean leaf was used
to isolate each female (one arena per leaf). After being on the residues for 1, 2, 3 or 4 d the 20 females were transferred to unsprayed arenas for 14 d. Fresh leaves were supplied every 2 d. Conditions after treatment were similar to those described for the previous experiments. The fecundity of individual females and egg hatch was assessed daily. Each female was considered as a replicate.

**Population Development.** To provide an indication of the effect of thuringiensin on development of *T. urticae* the instantaneous mortality rate (Krebs 1985) was calculated for mites exposed to four concentrations (0.00015, 0.0003, 0.003, 0.03 g AI/litre). Thuringiensin was applied to whole French dwarf bean leaves with a Potter tower, and a control treated with water was also established. Ten females were placed into 12 mm diameter arenas for 24 h. At least 200-250 eggs were laid by *T. urticae* females per treatment. Each arena was considered as a replicate; 10 replicates were used for each concentration tested. Leaves were held at 23 ± 2°C and a photoperiod of 16:8(L:D) until instantaneous mortality rates were assessed. Individuals were considered dead when they did not proceed through to the succeeding chrysalid stage at a time equivalent to those in the control groups. A daily evaluation of mortality or unsuccessful moulting was done up to the time the control treatment reached the adult stage (7 d). The instantaneous mortality rate \(i\) was calculated for each treatment (egg to adult) with the formula \(i = (\ln N_t - \ln N_0)/t\), where \(\ln N_t = \) natural log of final total population, \(\ln N_0 = \) natural log of
initial population and $t$ = time that elapsed between the initial and final population (7 d). In addition, the percentage of real mortality was calculated on the basis of the initial number of eggs and the partial mortality of each stage for each concentration (Southwood 1978).

**Statistical Analysis.** The responses of the test subjects to different thuringiensin concentrations were analysed by log-probit analysis (POLO; Russell et al. 1977). The same program was used to test the goodness-of-fit to the probit model based on a $\chi^2$ goodness-of-fit test, and the hypotheses of equality (slopes and intercepts of two regressions are equal) and parallelism (slopes of two regressions are equal) were also tested. Statistical differences between LC values were measured by using a 95% confidence interval (CI) for the ratio of two values Robertson & Preisler (1992). When comparison of the differences between treatments in other experiments was necessary, the natural or transformed data (arcsin $\sqrt{\%}$) were subjected to one- or two-way ANOVA, and the means were compared by Tukey's test (Zar 1984).

**Results**

For both *T. urticae* and *P. ulmi*, the responses of each developmental stage were compared to determine their relative susceptibilities to thuringiensin through direct or residual exposure.
**Direct Toxicity of Thuringiensin to T. urticae.** Effects of direct sprays on the mortality of larvae, protonymphs, deutonymphs and adults are shown in Table 1 and Figure 1. Regressions for larvae and protonymphs were equal. However, the hypothesis of equality was rejected when the regressions for larvae and protonymphs were compared against those for deutonymphs and adults. The hypothesis of parallelism was not rejected for regressions of larvae compared with those for adults.

The relative susceptibility of T. urticae life stages was calculated only when ratios of LC$_{50}$ or LC$_{90}$ were significantly different at the 95% confidence limits (Robertson & Priesler 1992). At the LC$_{50}$, larvae and protonymphs were significantly more susceptible to thuringiensin than deutonymphs (6-fold) and adults (488-fold). At the LC$_{90}$, larvae and protonymphs were significantly more susceptible to thuringiensin than were deutonymphs (16-fold) and adults (530-fold).

**Direct Toxicity of Thuringiensin to P. ulmi.** Effects of direct sprays on the mortality of larvae, protonymphs, deutonymphs and adults are shown on Table 2 and Figure 2. Hypotheses of equality and parallelism between regressions were rejected for comparisons of all stages. The estimated LC$_{50}$ and LC$_{90}$ for larvae were significantly different from all other stages at the 95% confidence limits (Robertson & Priesler 1992). At the LC$_{50}$, larvae were significantly more susceptible to
Table 1. Direct and residual toxicity of thuringiensin to *T. urticae* life stages.

<table>
<thead>
<tr>
<th>Stage tested</th>
<th><em>LC$_{50}$</em> g AI/litre</th>
<th>95% CI</th>
<th><em>LC$_{90}$</em> g AI/litre</th>
<th>95% CI</th>
<th>Slope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>larva</td>
<td>901</td>
<td>0.0011</td>
<td>0.0006-0.0016</td>
<td>0.0054</td>
<td>0.0037-0.0102</td>
</tr>
<tr>
<td>protonymph</td>
<td>860</td>
<td>0.0010</td>
<td>0.0004-0.0019</td>
<td>0.0050</td>
<td>0.0025-0.0187</td>
</tr>
<tr>
<td>deutonymph</td>
<td>1752</td>
<td>0.0066</td>
<td>0.0032-0.0185</td>
<td>0.0887</td>
<td>0.0274-1.8700</td>
</tr>
<tr>
<td>adult</td>
<td>313</td>
<td>0.5360</td>
<td>0.3514-0.7372</td>
<td>2.8614</td>
<td>1.7875-7.3809</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>larva</td>
<td>745</td>
<td>0.0028</td>
<td>0.0020-0.0036</td>
<td>0.0076</td>
<td>0.0054-0.0146</td>
</tr>
<tr>
<td>protonymph</td>
<td>661</td>
<td>0.0138</td>
<td>0.0104-0.0175</td>
<td>0.0521</td>
<td>0.0395-0.0768</td>
</tr>
<tr>
<td>deutonymph</td>
<td>1002</td>
<td>0.0654</td>
<td>0.0092-0.3489</td>
<td>1.8221</td>
<td>0.3432-223.91</td>
</tr>
<tr>
<td>adult</td>
<td>580</td>
<td>2.7886</td>
<td>0.3974-9.6972</td>
<td>41.064</td>
<td>11.189-15372.</td>
</tr>
</tbody>
</table>

*LCs estimated by probit analysis (POLO; Russell et al. 1977).

Table 2. The direct toxicity of thuringiensin to *P. ulmi* life stages.

<table>
<thead>
<tr>
<th>Stage tested</th>
<th><em>LC$_{50}$</em> g AI/litre</th>
<th>95% CI</th>
<th><em>LC$_{90}$</em> g AI/litre</th>
<th>95% CI</th>
<th>Slope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>larva</td>
<td>1163</td>
<td>0.00002</td>
<td>0.000019-0.00002</td>
<td>0.00004</td>
<td>0.00003-0.00004</td>
</tr>
<tr>
<td>protonymph</td>
<td>986</td>
<td>0.00006</td>
<td>0.00005-0.00007</td>
<td>0.00017</td>
<td>0.00014-0.00021</td>
</tr>
<tr>
<td>deutonymph</td>
<td>1201</td>
<td>0.00018</td>
<td>0.00013-0.0002</td>
<td>0.00068</td>
<td>0.0005-0.0011</td>
</tr>
<tr>
<td>adult</td>
<td>930</td>
<td>0.07093</td>
<td>0.03013-0.12359</td>
<td>0.88954</td>
<td>0.49035-2.37990</td>
</tr>
</tbody>
</table>

*LCs estimated by probit analysis (POLO; Russell et al. 1977).
Fig. 1. The responses of *T. urticae* stages to direct sprays of thuringiensin.

<table>
<thead>
<tr>
<th>Larva</th>
<th>Protonymph</th>
<th>Deutonymph</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>8.0</td>
<td>5.5</td>
<td>4.5</td>
</tr>
<tr>
<td>6.0</td>
<td>5.5</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>5.5</td>
<td>5.0</td>
<td>4.5</td>
<td>3.5</td>
</tr>
<tr>
<td>5.0</td>
<td>4.5</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4.5</td>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>4.0</td>
<td>3.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Log. concentration g (Al)/l

Percent mortality

Probit

Log. concentration g (Al)/l
Fig. 2. The responses of *P. ulmi* stages to direct sprays of thuringiensin.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva</td>
<td></td>
</tr>
<tr>
<td>Protonymph</td>
<td></td>
</tr>
<tr>
<td>Deutonymph</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td></td>
</tr>
</tbody>
</table>

Log. concentration g (AI)/L
thuringiensin than protonymphs (3-fold), deutonymphs (9-fold) and adults (3546-fold). At the LC$_{90}$, larvae were significantly more susceptible to thuringiensin than protonymphs (4-fold), deutonymphs (17-fold) and adults (22.3x$10^3$-fold).

**Residual Toxicity of Thuringiensin to *T. urticae*.** Effects of exposure of *T. urticae* to various residues of thuringiensin are shown in Table 1 and Figure 3. The hypothesis of equality was rejected for all stages, whereas the hypothesis of parallelism was not rejected for comparisons between the regressions for larvae and protonymphs and between deutonymphs and adults. At the LC$_{50}$, *T. urticae* larvae were significantly more susceptible to thuringiensin than were protonymphs (5-fold), deutonymphs (23-fold) and adults (996-fold). At the LC$_{90}$, larvae were significantly more susceptible to thuringiensin than were protonymphs (7-fold), deutonymphs (240-fold) and adults (5,403-fold).

**Separation of Direct, Residual and Feeding Toxicity.** Table 3 shows that mortality of *T. urticae* larvae exposed to direct sprays, residues and residues-only was higher on Petri dishes than on leaves. The combined action of direct sprays and exposure to residues produced the highest mortality on both substrates. Although feeding was possible on leaves definite conclusions about oral toxicity were not possible because, when feeding was excluded by placing larvae on residues in Petri dishes, higher mortality occurred (54.7%) on this substrate than on leaf
Fig. 3. The responses of *T. urticae* stages to residues of thuringiensin.

Larva
Protonymph
Deutonymph
Adult
Table 3. The effect of direct, residual, and feeding toxicity of thuringiensin (0.005 g AI/litre) to _T. urticae_.

<table>
<thead>
<tr>
<th>Substrate and Treatment</th>
<th>Mean % Mortality (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petri dish (no feeding possible)</td>
<td></td>
</tr>
<tr>
<td>Direct+Residue</td>
<td>120</td>
</tr>
<tr>
<td>Residue</td>
<td>125</td>
</tr>
<tr>
<td>Direct</td>
<td>120</td>
</tr>
<tr>
<td>Control (water)</td>
<td>130</td>
</tr>
<tr>
<td>Leaf (feeding possible)</td>
<td></td>
</tr>
<tr>
<td>Direct+Residue</td>
<td>125</td>
</tr>
<tr>
<td>Residue</td>
<td>120</td>
</tr>
<tr>
<td>Control (water)</td>
<td>125</td>
</tr>
</tbody>
</table>

ANOVA was done using untransformed % mortality values.

* Values with same letters indicate means are not significantly different ($P > 0.05$, Tukey's test [Zar 1984]).
surfaces where feeding was possible (23%).

**Fecundity and Egg Hatching.** Table 4 shows that, after 2 d exposure to thuringiensin, the fecundity of females exposed to residues only was reduced significantly ($P < 0.05$); however, the proportion of eggs hatching was not affected compared with hatch observed in the control. Similar results were obtained when females were sprayed directly and remained on residues for similar periods. Fecundity was not significantly ($P > 0.05$) different between mites directly sprayed and those that were exposed to residues, with the exception of the 3 d exposure treatment. For this exposure, significantly ($P < 0.05$) fewer eggs were laid by females exposed to direct sprays and residues.

**Population Development.** Table 5 shows that thuringiensin caused increases in the instantaneous mortality rate ($i$) of *T. urticae*; values of ($i$) were 13.1-, 33.6- and 125-fold higher compared with the control at 0.00015, 0.0003 and 0.003 g AI/litre, respectively. Table 5 indicates that larvae suffered the highest real mortality for all concentrations. Total real mortality ranged between 18.4-100% from the lowest to highest thuringiensin concentration.
Table 4. Effect of different periods of exposure to residues and direct spraying of thuringiensin on egg laying and hatching of *T. urticae*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exposure to residue only</th>
<th>Exposure to direct+residue</th>
<th>Comparison of mean egg%d between exposure methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean eggs/♀/d (±SEM)</td>
<td>% Hatch (±SEM)</td>
<td>Mean eggs/♀/d (±SEM) % Hatch (±SEM)</td>
</tr>
<tr>
<td>Untreated</td>
<td>8.64 (0.91)a*</td>
<td>90.0 (5.78)a</td>
<td>7.04 (1.17)a 92.7 (4.12)a **</td>
</tr>
<tr>
<td>1 d exposure</td>
<td>7.06 (2.13)ab</td>
<td>90.1 (5.21)a</td>
<td>5.27 (1.98)ab 84.4 (12.43)a</td>
</tr>
<tr>
<td>2 d exposure</td>
<td>5.19 (1.87)bca</td>
<td>87.8 (7.45)a</td>
<td>3.44 (2.10)b 68.0 (24.39)a</td>
</tr>
<tr>
<td>3 d exposure</td>
<td>4.01 (1.22)cde</td>
<td>85.2 (8.61)a</td>
<td>1.06 (1.54)c 70.7 (17.00)a</td>
</tr>
<tr>
<td>4 d exposure</td>
<td>0.61 (0.98)e</td>
<td>74.2 (20.8)a</td>
<td>0.34 (1.08)e 74.5 (33.22)a</td>
</tr>
</tbody>
</table>

* Values with same letters sign indicate means are not significantly different (*P* > 0.05, Tukey's test [Zar 1984]).

** t-test (*P* = 0.05). Symbol (=) indicates no significant difference between exposure methods.

Table 5. The effect of thuringiensin on *T. urticae* population mortality.

<table>
<thead>
<tr>
<th>Thuringiensin concentration g AI/litre</th>
<th>Number of each life stage surviving (% real mortality of each life stage)</th>
<th>Instantaneous mortality rate (i)</th>
<th>Total % real mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eggs</td>
<td>larva</td>
<td>proto</td>
</tr>
<tr>
<td>Control</td>
<td>256</td>
<td>253</td>
<td>253</td>
</tr>
<tr>
<td>0.00015</td>
<td>(1.2)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td></td>
<td>(12.6)</td>
<td>(5.3)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>0.0003</td>
<td>270</td>
<td>260</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>(3.7)</td>
<td>(29.3)</td>
<td>(7.8)</td>
</tr>
<tr>
<td>0.003</td>
<td>262</td>
<td>260</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>(0.8)</td>
<td>(50.8)</td>
<td>(32.4)</td>
</tr>
<tr>
<td>0.03</td>
<td>201</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(99.5)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Slow action of thuringiensin has been accepted as a typical feature of this toxicant (Krieg 1968; Hall et al. 1971; Perring & Farrar 1986; Neal et al. 1987; Royalty & Perring 1987; Royalty et al. 1990, 1991). However, the experiments in this study showed unexpected results that differed from those previously described in the literature.

Although the assessment of larval mortality was done after 120 h, (i.e., when mites in the control group reached adulthood), actual mortality is likely to have occurred at moulting when larvae were due to enter the next stage. Similar delayed mortality was noticed when T. urticae larvae and nymphs were treated with hexythiazox (Welty et al. 1988, Chapman & Penman, 1988) and when P. citri were treated with cycloprate (Asano & Kamei, 1977). Such a delayed effect is an important feature of thuringiensin toxicity because, for larvae, mortality occurs on average 24-36 h after exposure. The mortality assessments for other immature stages were also done when mites in the control group reached adulthood. On average mortality of protonymphs and deutonymphs occurred 24-48 h after exposure to thuringiensin, reflecting the slightly longer developmental period for these stages. By comparison, mortality of adults was considerably slower and adults could survive for up to 2 weeks after treatment. Therefore, adult mortality might be a consequence of the disruption of a different biochemical mechanism to that in juvenile mites (Beebee & Bond 1973 a, b).

To avoid misinterpretation on the toxicological effects of
chemicals that affect the metabolic processes involved in moulting, use of an appropriate mortality assessment criterion and an accurate concentration-response range are necessary. Identification and separation of the mortality that occurs at moulting of the stage exposed to the chemical from that which occurs throughout development up to the adult stage by successive, and probably, cumulative intake of the chemical is also necessary.

The results of this study strongly suggest that thuringiensin may achieve effective control of immature mite stages in a relatively short time, a conclusion which is in disagreement with information previously cited about the slow activity of thuringiensin on *T. urticae* and *P. ulmi*. (Royalty et al. 1990, 1991).

The stage of immature development had previously been shown to be not important in the mortality responses of *T. urticae* (Royalty et al. 1990), however, this study has shown that regressions for residual exposure for all stages were significantly different and that the LC values increased with the developmental stage (Table 1, Figure 3). This trend was less evident with direct exposure because the responses of larvae and protonymphs were similar (Table 2, Figure 2). However, these results clearly demonstrate that early immature stages are more susceptible to thuringiensin than are the later immatures and adults. With *P. ulmi* LC values significantly increased with the developmental stage, a result which also differs from those presented by Royalty et al. (1991). Possibly the differences found between our results and those of Royalty
et al. (1990, 1991) were due to different bioassay methods, formulations, exposure times, mortality assessment intervals, ages of immature mites or combination of any or all of these factors.

Robertson & Worner (1990) suggested that separation of the effects of the toxicant route through residues, direct and feeding should be considered when a pesticide is being tested. Thuringiensin has been tested for each exposure route in other studies, but no conclusive results have been obtained (Neal et al. 1987; Royalty et al. 1990, 1991); however, Sebesta et al. (1969) mentioned that in insects thuringiensin was less toxic perorally than parentally. Results for T. urticae show significant differences between the slopes and LC values for direct and residual exposure for different instars (Figures 1 and 3). The LC ratios for immature stages between direct and residual exposure increase with the developmental stage, suggesting that the greatest susceptibility occurs when larvae are directly sprayed with thuringiensin. This study has also demonstrated that direct and residual effects do not act independently (Table 3); thus, an interaction might occur among direct, residual and feeding toxicity. These results suggest that the combined effects of direct, residual and feeding exposure would only be ensured under field conditions if efficient spray coverage of foliage was achieved.

The effectiveness of thuringiensin varied markedly between P. ulmi and T. urticae (Tables 1 and 2). LC$_{50}$ and LC$_{90}$s for each P. ulmi instar were significantly lower than the corresponding values for T. urticae for direct exposure. These differences
probably reflect a different sensitivity of DNA-dependent RNA polymerase (Beebee & Bond 1973 a,b). In addition, different degrees of susceptibility to thuringiensin may be due to a different ratio of thuringiensin to ATP at specific developmental stages among species (Sebesta 1969). However, other factors such as mite behaviour, poison penetration, physiological and biochemical differences could also account for susceptibility differences between the two species (Robertson & Priesler 1992, Holland 1992).

Exposure of *T. urticae* to residues or direct sprays significantly affected fecundity, which was inhibited by > 90% after 4 d (Table 4). This suggests that, in practice, thuringiensin may compensate for its low toxicity to adults with high inhibition of fecundity. The maximum reduction of fecundity for *T. urticae* recorded by other workers was 25% (Royalty et al. 1990). Again, different bioassay methods used to evaluate fecundity might account for the differences, because Royalty et al. (1990) evaluated the fecundity of the *F*₁ generation exposed to residues. A physiological basis for reduced fecundity has not been determined, and further studies may be necessary to fully exploit the process of inhibition of fecundity.

Up to 100% inhibition of the development of motile stages was achieved in one generation of *T. urticae* (Table 5). These results show that a significant reduction of the mite population occurred beyond the stage that was initially exposed to the chemical. As indicated by Robertson & Worner (1990), population toxicology is far more realistic than prediction based on the
response of anyone stage. Therefore, because the field rate
could be higher than the concentration levels tested here and
that thuringiensin residues have a relatively long period of
activity, the potential for suppression of field populations
seems likely to be high. Furthermore, prolonged residual
activity could offset the lack of ovicidal effects of
thuringiensin because larvae would be exposed to a lethal
concentration when they hatch.

Overall, this study suggests that thuringiensin would be an
effective acaricide against immature *T. urticae* and *P. ulmi* and
could reduce populations in a relatively short time either by
exposure to direct sprays or residues. Considerable reductions
in fecundity may also be achieved by these routes of exposure.
However, field experiments with different population stage
structures would be necessary to determine the ideal spray
timing for both spider mite species. Ideally, the phenology of
each species could then be used as the basis for application of
thuringiensin on a field population (Robertson & Worner 1990).
Chapter 3

Factors Influencing the Responses of Tetranychus urticae Koch (Acarina: Tetranychidae) to Thuringiensin.

Introduction

THURINGIENSIN, a $\beta$-exotoxin of Bacillus thuringiensis, has been reported to have significant potential for control of mites (Grau, 1986; Neal et al. 1987; Perring & Farrar, 1986; Hoy & Ouyang, 1987; Royalty et al. 1990, 1991), however, limited activity against adults and lack of ovicidal action could restrict its usefulness. Because several environmental and biological factors (e.g., temperature, humidity, host plant, surfactant, age of test subjects) may influence the response of a test organism to a pesticide, the factors that influence thuringiensin toxicity should be identified to maximise its effectiveness.

Temperature is one of the most important factors influencing the response of arthropods to pesticides. Miticides such as cyhexatin, dicofol, chlorobenzilate and propargite are
all reported to have a positive temperature-toxicity correlation (Fisher & Hansell 1964; Stenseth 1976; Everson & Tonks 1981; James et al. 1988). In contrast, the responses to dicofol and tetryadifon were negatively correlated with temperature (Hassan et al. 1970). No information about the effects of temperature on the response of spider mites to thuringiensin has been yet been published.

The response of arthropods to pesticides can also be influenced by the host plant. For example, Wakou & Sugawara (1974) found that the response of *T. urticae* eggs to dicofol on peach, bean and apple leaves was affected by the relationship between leaf surface structure and the amount of chemical deposited. Asano & Kamei (1982) also showed that responses of *Panonychus citri* (McGregor) and *T. urticae* eggs to cycloprate varied with different host plants tested. Similarly Marris & Chapman (1987) found that hexythiazox caused greatest mortality to *T. urticae* eggs laid on broad bean (*Vicia faba*) leaves and lowest mortality to eggs laid on apple (*Malus* sp.) leaves. An intermediate level of mortality was observed for eggs laid on raspberry (*Rubus ideaus*) and strawberry (*Fragaria ananassa*) leaves. In contrast to these studies with synthetic pesticides no specific studies on the effect of host plant on thuringiensin toxicity have been reported.

To maximise the effectiveness of a pesticide, mobility through the plant or over the surface is often desirable. Munthali & Wyatt (1986) demonstrated the significance of this aspect of efficacy when they reported that more *T. urticae* eggs were killed when dicofol was transported across the leaf surface
than when it was directly deposited on the eggs. Hexythiazox, an ovicidal miticide, also has been shown to have mobility on the leaf surface (Anon. 1984). Although Mersie & Singh (1988) showed that thuringiensin was absorbed to a limited extent by snapbean (*Phaseolus vulgaris*, cv., Greencrop) leaves, Neal et al. (1987) reported that thuringiensin was not translocated in bush lima bean (*Phaseolus lunatus*). Further study on the mobility and subsequent effects of thuringiensin in and on spider mite host plants is therefore warranted.

The mobility of a pesticide on a plant surface may also be influenced by surfactants that are present in formulations or added to spray mixes. Many investigators (e.g., Stevens et al. 1988, Buick et al. 1990, Dentener & Peetz 1992) have shown that surfactants frequently increase the spread and foliar absorption of pesticides, growth regulators and nutrients on leaves. As with other factors that may influence the toxicity of thuringiensin, few studies have been done with surfactants and thuringiensin. One study of thuringiensin uptake showed that surfactant (X-77) did not markedly increase C14-labelled thuringiensin penetration into the leaf of snapbean (Mersie & Singh 1988).

Persistence of residues also significantly affects the efficacy of many pesticides. Wolfenbarger et al. (1972) and Mersie & Singh (1988) reported that detectable residues of thuringiensin persisted for 7 d on snapbeans and 12 d on cotton (*Gossypium hirsutum* L.) leaves. Hall et al. (1971) showed that thuringiensin was toxic to *P. citri* for at least 45 d on orange (*Citrus sp.*, cv., 'Valencia'). Royalty et al. (1990, 1991)
reported that *T. urticae* on lima bean (*Phaseolus lunatus* L.) and *P. ulmi* on apple (cv., 'Red Delicious') were most susceptible to thuringiensin 12 d after treatment. Neal et al. (1987) demonstrated that a high degree of residual activity of thuringiensin against *T. urticae* (76.6% mortality) and *T. cinnabarinus* (Boisduval) (86.7% mortality) occurred after 12 d on lima bean. Therefore the residual activity of thuringiensin is apparently quite variable and may be strongly influenced by the host plant.

Finally, the age of test subjects used in bioassays may markedly influence the response of an organism to a pesticide. The toxicity of thuringiensin (Royalty et al. 1990, 1991) and other pesticides that affect juvenile mite development has been estimated only for specific instars (Ebling & Pence 1954; Aveyard et al. 1986; Welty et al. 1988; Marshall & Pree 1991); no reference has been found indicating different instar-age-responses to miticides. Information on the age-specific susceptibility of immature stages would be useful for improving the reliability of bioassays, especially those involving miticides that disrupt the moulting of juvenile mites.

The main objectives of this study were to investigate the influence of temperature, host-plant, active ingredient mobility, surfactant, residue age and larval age on the responses of *T. urticae* to thuringiensin.
Materials and Methods

Sources of Mites. A *T. urticae* strain has been maintained by the Department of Entomology, Lincoln University, Canterbury since 1985 without exposure to pesticides. Mites were reared on French dwarf bean (*Phaseolus vulgaris*, cv. 'Tendergreen') in the laboratory; the photoperiod was 16:8 (L:D). Temperature and humidity were not controlled but were approximately 21 ± 3°C and 60 ± 15% (RH). New plants were added to the colony when required.

Miticide. An experimental formulation of thuringiensin (ABG-6320), an aqueous suspension (5% AI) supplied by Abbott Laboratories, USA, was used in all experiments. The physical and chemical properties of thuringiensin were previously reported by Sebesta et al. (1981).

Bioassays. Unless otherwise noted, a Potter tower (Burkard Manufacturing Co. Ltd, Rickmansworth, U.K.) was used for applying thuringiensin suspensions in all experiments. Two ml of thuringiensin suspension was sprayed by the Potter tower on each occasion at 55 ± 5 kPa; a 10 s settling period followed. This resulted in a wet deposit of 1.25 ± 0.01 mg/cm² (the deposit was estimated by weighing aqueous deposits on microscope coverslips).

When a concentration-mortality response was to be estimated assuming the probit model, preliminary experiments with a small number of mites were done to select a series of five
concentrations that would produce 5–95% mortality (Robertson et al. 1984). Except for those that were used to determine the effect of larval age on the response to thuringiensin, larvae selected were approximately half-way through their development.

Whole-leaf, twin-leaf and leaf disc methods were used in the bioassays. With the whole-leaf method, French dwarf bean leaves were placed upside down on moistened cotton wool in Petri dishes (85 mm diameter). Run-off from the leaves was prevented by confining mites in 12 mm diameter arenas surrounded by a sticky insect trap adhesive (Davis Gelatine N.Z. Ltd, Christchurch). On experiments in which leaf discs were used, five discs (12 mm diameter) were placed on moistened cotton wool in a Petri dish. With the twin-leaf method, French dwarf bean plants at the two-leaf stage were cut at their stem base and placed in vials (2.5 x 7.5 cm) filled with water; only when roots had developed were the twin leaves considered suitable for use. Run-off from the leaves was prevented by confining mites in arenas (12 mm diameter) on the undersides of leaves as described for the whole-leaf method. In all experiments, mites were held at 23 ± 2°C and a photoperiod of 16:8(L:D) until the control group mites reached adulthood. Assessment of mortality was done daily; mites were considered dead when individuals did not reach the succeeding chrysalid stage during a time equivalent to those in the control groups.

**Temperature.** To determine the effect of temperature on the responses of *T. urticae* larvae to thuringiensin, five concentrations of thuringiensin 5% SC were applied to whole bean
leaves. A water-treated control was used. Applications were made from the lowest to highest thuringiensin concentrations after the control groups were treated with water only. At least 25 larvae were transferred into each arena; each concentration tested was replicated four times (one replicate per leaf). Leaves were held at 13, 18, 23 and 28 ± 2°C and a photoperiod 16:8(L:D) until the control group mites reached adulthood. The responses of the larvae held at different temperatures to residues of thuringiensin were analysed by probit analysis (POLO; Russell et al. 1977). The same program was used for testing goodness-of-fit to the probit model by the $\chi^2$ goodness-of-fit test. The hypothesis of equality (slopes and intercepts of two regressions are equal) and paralellism (slopes of two regressions are equal) were also tested. Statistical differences between LC values were evaluated on the basis of 95% confidence intervals (CI) for the ratio of two values (Robertson & Preisler 1992).

The interaction between temperature and concentration was studied in a separate experiment where three concentrations of thuringiensin (0.00015; 0.003; 0.03 g AI/litre) were applied to whole French dwarf bean leaves. A control treated with water was also included. At least 25 larvae were transferred into arenas; four replicates for each treatment were included. Leaves were held at 13, 18 and 23 ± 2°C and a photoperiod of 16:8(L:D) until the control group mites reached adulthood. To compare the differences between treatments, transformed data (arcsin $\sqrt{\%}$) were subjected to a two-way ANOVA. The means were compared with Tukey's test (Zar 1984).
Host Plant. The influence of host plant on the response of T. urticae to thuringiensin was studied in three separate experiments. The effects of leaf type and leaf surface (lower and upper) were tested by applying one concentration (0.00025 g AI/litre) of thuringiensin 5% aqueous suspension to French dwarf bean, peach (Prunus persica, cv. 'Red Haven') and apple (Malus sp. cv. 'Red Delicious') leaf discs. A control with water was also established for each leaf type. At least 25 larvae were transferred to each leaf disc; four replicates per treatment were included. Leaf discs were held at 23 ± 2°C and a photoperiod of 16:8(L:D) until the control group mites reached adulthood. To compare the differences between treatments, transformed data (arcsin √%) were subjected to a two-way ANOVA. The means were compared with Tukey's test (Zar 1984).

Translocation. The translaminar effect of thuringiensin were tested with the twin-leaf method. To test for a translaminar effect, two thuringiensin concentrations (0.0828 and 8.28 g AI/litre) were applied. The upper surfaces were lightly brushed with each thuringiensin suspension to which Citowett surfactant (0.025% alkylaryl polyglycol ether) had been added to improve retention. Approximately 0.16 ml was applied per leaf. Six-twelve-h-old larvae were placed into arenas (12 mm diam.) on the lower surface of the leaves and were held at 23 ± 2°C and a photoperiod of 16:8(L:D) until the control group mites reached adulthood.

The systemic effect of thuringiensin was investigated using the twin-leaf method. One leaf was dipped in a suspension of
thuringiensin (8.28 g AI/litre) + Citowett (0.025%). Approximately 0.21 ml was applied per leaf. Six-twelve-h-old larvae were placed into arenas on the lower side of the untreated leaf opposite the treated leaf. For both experiments at least 20 larvae were used per replicate and there were six replicates for each treatment. Conditions after treatment and evaluations were similar to those already described. Transformed data (arcsin $\sqrt{n}$) were subjected to one-way ANOVA and means were separated by a t-test or Tukey's test.

**Surfactant.** The organosilicone Silwet L-77 (oxyethylene methyl siloxane) was used in this experiment because it has been shown to have surfactant properties superior to non-organosilicone surfactants (Stevens et al. 1988, Buick et al. 1990). After preliminary tests, single Silwet L-77 (0.1% v/v) and thuringiensin (0.00025 g AI/litre) concentrations were selected. Whole French dwarf bean leaves were sprayed and after the residues had dried at least 25 larvae were transferred into arenas. A water-treated control was also established and four replicates were used for each treatment. Leaves were held at 23 ± 2°C and a photoperiod of 16:8(L:D) until the control group mites reached adulthood. Transformed data (arcsin $\sqrt{n}$) were subjected to one-way ANOVA and the means were compared with Tukey's test (Zar 1984).

**Residue Age.** To determine the responses of larvae to thuringiensin residues of different ages, five concentrations of thuringiensin 5% SC were applied to whole bean leaves. A
control treated with water was also used. Treated leaves were held in the controlled temperature cabinet at 23 ± 2°C and a photoperiod of 16:8(L:D). Larvae were transferred to arenas on treated leaves at 1, 2, 4, 8, 13 and 21 d after application. At least 25 larvae were transferred to each arena; four replicates for each concentration were tested. Leaves were held at 23 ± 2°C and a photoperiod 16:8(L:D) until the control group mites reached adulthood. The responses of larvae to thuringiensin residues of different ages were analysed by probit analysis as described above.

**Larval Age.** To determine the responses of larvae of different ages to thuringiensin residues, five concentrations were applied to whole French dwarf bean leaves. Larvae of three ages (2, 12, 18 h) were placed on the residues for 6 h and then transferred to leaves free of residues. A control treated with water was also included. At least 25 larvae were transferred into arenas; four replicates for each larval age were tested. The responses of larvae of different ages to thuringiensin residues were analysed by probit analysis as described previously.

The interaction between larval age and thuringiensin concentration was investigated in a separate experiment in which three thuringiensin concentrations (0.0002, 0.001, 0.005 g AI/litre) and two larval ages (2 and 10 h) were used. Larvae were placed on residues for 8 h, then transferred to residue-free leaves and held at 23 ± 2°C and a photoperiod of 16L:8D until the control group mites reached adulthood. A control
group treated with water was also used. Replications of at least 25 larvae were included for each treatment. To compare the differences between treatments, transformed data (arcsin $\sqrt{\%}$) were subjected to two-way ANOVA. The means were compared using Tukey's test (Zar 1984).

**Results**

**Temperature.** The responses of *T. urticae* larvae to thuringiensin residues at different temperatures are shown in Table 1 and Figure 1. Only regressions for 13 and 18°C were equal. The hypothesis of parallelism was not rejected for regressions between 13 and 28°C, and between 18 and 28°C. The relative susceptibility of larvae was calculated only when the ratios of the LC$_{50}$ or LC$_{90}$ were significantly different. At the LC$_{50}$, larvae that were exposed to thuringiensin residues at 13 and 18°C were significantly more susceptible than were those larvae held at 23°C (6-fold) and 28°C (5-fold). At the LC$_{90}$, larvae that were exposed to thuringiensin residues at 13 and 18°C were 5-fold more susceptible than those that were held at 23 and 28°C. These results indicate that thuringiensin toxicity significantly increased with decreasing temperature. Table 2 shows that temperature and thuringiensin concentration had a significant ($P = 0.05$) effect on larval mortality and that the interaction between temperature and thuringiensin concentration was significant ($P = 0.01$)

**Residue Age.** The responses of *T. urticae* larvae to
Table 1. The effect of temperature after treatment on the toxicity of thuringiensin to *T. urticae* larvae.

<table>
<thead>
<tr>
<th>°C</th>
<th>n</th>
<th>LC$_{50}$*</th>
<th>95% CI</th>
<th>LC$_{90}$*</th>
<th>95% CI</th>
<th>Slope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g AI/litre)</td>
<td></td>
<td>(g AI/litre)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1,053</td>
<td>0.00031</td>
<td>0.00021-0.00044</td>
<td>0.00103</td>
<td>0.00069-0.00214</td>
<td>2.45 (0.14)</td>
</tr>
<tr>
<td>18</td>
<td>1,114</td>
<td>0.00037</td>
<td>0.00023-0.00056</td>
<td>0.00116</td>
<td>0.00073-0.00281</td>
<td>2.57 (0.14)</td>
</tr>
<tr>
<td>23</td>
<td>901</td>
<td>0.00190</td>
<td>0.00064-0.00164</td>
<td>0.00542</td>
<td>0.00327-0.01438</td>
<td>1.84 (0.13)</td>
</tr>
<tr>
<td>28</td>
<td>999</td>
<td>0.00137</td>
<td>0.00084-0.00205</td>
<td>0.00504</td>
<td>0.00314-0.01268</td>
<td>2.26 (0.13)</td>
</tr>
</tbody>
</table>

*LCs estimated by probit analysis (POLO; Russell et al. 1977).

Table 2. The response of *T. urticae* larvae to three thuringiensin concentrations at three different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Concentration (g AI/litre)</th>
<th>Mean % mortality (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.00015</td>
<td>21.5 (± 4.72)</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>44.5 (± 22.5)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>97.5 (± 0.57)</td>
</tr>
<tr>
<td>18</td>
<td>0.00015</td>
<td>5.25 (± 2.99)</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>46.8 (± 18.28)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>93.3 (± 7.41)</td>
</tr>
<tr>
<td>23</td>
<td>0.00015</td>
<td>4.75 (± 1.5)</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>36.25 (± 21.82)</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>55.75 (± 9.95)</td>
</tr>
</tbody>
</table>

* Mortality data transformed to arcsin $\sqrt{\%}$. *Values with same letters indicate no significant difference ($P > 0.05%$; Tukey's test [Zar 1984]).

ANOVA - concentration ($F = 110.6 ; df = 2,27; P = 0.05$); temperature ($F = 13.4; df = 2,27; P = 0.05$); concentration x temperature ($F = 4.6; df = 4,27; P = 0.01$).
Fig. 1. The response of *T. urticae* larvae to thuringiensin at different temperatures.

13 degrees
18 degrees
23 degrees
28 degrees

% Mortality

Log. concentration g (Al)/l
thuringiensin residues of different ages are shown in Table 3 and Figure 2. Regressions for 4- and 8-d-old residues and for 13- and 21-d-old residues were equal. The hypothesis of parallelism was not rejected for all regressions, except between regressions for 4- and 8- and for 4- and 21-d-old residues. The relative susceptibilities of larvae to residues of different ages were calculated only when ratios of the LC$_{50}$ or LC$_{90}$ were significantly different. At the LC$_{50}$ and LC$_{90}$, larvae exposed to 1-d-old residues were 1.5-fold more susceptible than were larvae on 8-d-old residues; and 4-fold more susceptible than larvae on 13- and 21-d-old residues. These results show that the larval response to residues was relatively consistent up to 8 d, after which time the response declined.

**Leaf Type.** The responses of *T. urticae* larvae to thuringiensin residues on different leaf types are shown in Table 4. Larval mortality on bean leaf was significantly ($P < 0.05$) lower than on peach and apple. Mortality between leaf sides for any leaf type was not significantly different ($P > 0.05$).

**Translocation.** Table 5 shows that thuringiensin had no appreciable translaminar or systemic activity when tested at two concentrations equivalent to 30- and 3,000-fold of the LC$_{50}$ for larvae exposed to residues.

**Surfactant.** The effects of surfactant on the responses of *T. urticae* larvae to thuringiensin are shown in Table 6. Silwet
Fig. 2. The response of *T. urticae* larvae to thuringiensin residues of different ages.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Log. concentration (g Al/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One day</td>
<td>6.5</td>
</tr>
<tr>
<td>Two days</td>
<td>6.0</td>
</tr>
<tr>
<td>Four days</td>
<td>7.0</td>
</tr>
<tr>
<td>Eight days</td>
<td>5.5</td>
</tr>
<tr>
<td>Twenty one days</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Percent mortality plotted against log concentration g (Al)/l.
Table 3. The toxicity of thuringiensin to *T. urticae* larvae, exposed to residues of different ages.

<table>
<thead>
<tr>
<th>Residue age (d)</th>
<th>n</th>
<th>LC$_{50}$ (g AI/litre)</th>
<th>95% CI</th>
<th>LC$_{90}$ (g AI/litre)</th>
<th>95% CI</th>
<th>Slope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>611</td>
<td>0.00250</td>
<td>0.0023-0.0027</td>
<td>0.00568</td>
<td>0.00495-0.00673</td>
<td>3.55(0.25)</td>
</tr>
<tr>
<td>2</td>
<td>881</td>
<td>0.00335</td>
<td>0.0021-0.0048</td>
<td>0.01445</td>
<td>0.00930-0.03225</td>
<td>2.02(0.16)</td>
</tr>
<tr>
<td>4</td>
<td>803</td>
<td>0.00330</td>
<td>0.0018-0.0046</td>
<td>0.00904</td>
<td>0.00619-0.02412</td>
<td>2.92(0.86)</td>
</tr>
<tr>
<td>8</td>
<td>600</td>
<td>0.00370</td>
<td>0.0033-0.0042</td>
<td>0.00802</td>
<td>0.00697-0.00971</td>
<td>3.91(0.41)</td>
</tr>
<tr>
<td>13</td>
<td>871</td>
<td>0.01080</td>
<td>0.0071-0.01264</td>
<td>0.02319</td>
<td>0.01822-0.03727</td>
<td>3.58(0.30)</td>
</tr>
<tr>
<td>21</td>
<td>564</td>
<td>0.00982</td>
<td>0.0053-0.01378</td>
<td>0.01913</td>
<td>0.01366-0.06352</td>
<td>4.43(0.39)</td>
</tr>
</tbody>
</table>

LCs estimated by probit analysis (POLO; Russell et al. 1977).

Table 4. The response of *T. urticae* larvae to thuringiensin residues on different leaf types and surfaces.

<table>
<thead>
<tr>
<th>Leaf type</th>
<th>n</th>
<th>Mean % mortality (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lower</td>
<td>200</td>
<td>75.75 (3.3) a*</td>
</tr>
<tr>
<td>upper</td>
<td>198</td>
<td>72.00 (5.4) a</td>
</tr>
<tr>
<td>Peach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lower</td>
<td>195</td>
<td>95.50 (1.0) b</td>
</tr>
<tr>
<td>upper</td>
<td>201</td>
<td>97.00 (1.2) b</td>
</tr>
<tr>
<td>Apple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lower</td>
<td>201</td>
<td>94.50 (3.4) b</td>
</tr>
<tr>
<td>upper</td>
<td>198</td>
<td>95.00 (2.6) b</td>
</tr>
</tbody>
</table>

* ANOVA was done using Arccsin √%. Values with same letters indicate no significant difference (*P* > 0.05%; Tukey's test [Zar 1984]).
Table 5. Responses of *T. urticae* larvae to thuringiensin when tested for translaminar and systemic activity in French dwarf bean plants.

<table>
<thead>
<tr>
<th>Concentration (g AI/litre)</th>
<th>Test for translaminar action</th>
<th>Test for systemic action</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean % mortality (±SEM)</td>
</tr>
<tr>
<td>0</td>
<td>111</td>
<td>3.5 (3.8) a*</td>
</tr>
<tr>
<td>0.0828</td>
<td>109</td>
<td>2.6 (3.2) a</td>
</tr>
<tr>
<td>8.2800</td>
<td>114</td>
<td>2.8 (1.9) a</td>
</tr>
</tbody>
</table>

* ANOVA was done using Arcsin √%. Values with same letters indicate no significant difference (*P* > 0.05; [Zar 1984]).

** t-Test. Values with same letters indicate no significant difference (*P* > 0.05; [Zar 1984]).

Table 6. The effect of surfactant (Silwet L-77, 0.1% v/v) on the response of *T. urticae* larvae to thuringiensin residues (0.00025 g AI/litre).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean % Mortality (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>130</td>
<td>3.2 (1.5) a*</td>
</tr>
<tr>
<td>Surfactant</td>
<td>129</td>
<td>3.4 (2.1) a</td>
</tr>
<tr>
<td>Thuringiensin</td>
<td>126</td>
<td>15.4 (1.2) b</td>
</tr>
<tr>
<td>Surfactant + Thuringiensin</td>
<td>138</td>
<td>14.1 (7.4) b</td>
</tr>
<tr>
<td>Direct exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>150</td>
<td>0.1 (2.2) a</td>
</tr>
<tr>
<td>Surfactant</td>
<td>170</td>
<td>93.7 (4.8) d</td>
</tr>
<tr>
<td>Thuringiensin</td>
<td>168</td>
<td>57.6 (4.9) c</td>
</tr>
<tr>
<td>Surfactant + Thuringiensin</td>
<td>190</td>
<td>99.2 (0.9) d</td>
</tr>
</tbody>
</table>

* ANOVA was done using Arcsin √%. Values with same letters indicate no significant difference (*P* > 0.05%; Tukey's test [Zar 1984]).
L-77 did not significantly \((P > 0.05)\) affect mortality when larvae were exposed to thuringiensin residues or when they were sprayed directly. However, Silwet L-77 had a significant \((P < 0.05)\) contact effect when sprayed directly onto mites, thus confirming its miticidal properties (Dentener and Peetz 1992).

**Larval Age.** The responses of *T. urticae* larvae of different ages to thuringiensin residues are shown in Table 7 and Figure 3. The regressions for 12- and 18-h-old larvae were equal. The hypothesis of parallelism was not rejected between the regressions for 2- and 18-h-old and for 12- and 18-h-old larvae.

At the LC\(_{50}\), 2-h-old larvae were significantly less susceptible than were 12-h-old larvae (1.9-fold) and 18-h-old larvae (2.3-fold). At the LC\(_{90}\), *T. urticae* 2-h-old larvae were significantly less susceptible than were 12-h-old larvae (1.4-fold) and 18-h-old larvae (1.7-fold). Table 8 shows that both larval age and thuringiensin concentration had a significant \((P = 0.05)\) effect on larval mortality and that there was a significant \((P = 0.05)\) interaction between larval age and concentration. These data confirms the results in Table 7.

**Discussion**

Thuringiensin showed a negative temperature-toxicity relationship with larvae (Table 1); the interaction between temperature and concentration (Table 2) was significant. These results may reflect the underlying biochemical mechanism of
Table 7. The response of *T. urticae* larvae of different ages to thuringiensin residues.

<table>
<thead>
<tr>
<th>Larvae age (h)</th>
<th>n</th>
<th>LC$_{50}$* g AI/litre</th>
<th>95% CI</th>
<th>LC$_{90}$* g AI/litre</th>
<th>95% CI</th>
<th>Slope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>673</td>
<td>0.00211</td>
<td>0.00148-0.00278</td>
<td>0.00759</td>
<td>0.00533-0.01433</td>
<td>2.3(0.25)</td>
</tr>
<tr>
<td>12</td>
<td>900</td>
<td>0.00109</td>
<td>0.00064-0.00164</td>
<td>0.00542</td>
<td>0.00327-0.01438</td>
<td>1.8(0.13)</td>
</tr>
<tr>
<td>18</td>
<td>678</td>
<td>0.00090</td>
<td>0.00076-0.00107</td>
<td>0.00434</td>
<td>0.00338-0.00594</td>
<td>1.9(0.14)</td>
</tr>
</tbody>
</table>

*LCs estimated by probit analysis (POLO; Russell et al. 1977).*

Table 8. The effects of three thuringiensin concentrations on *T. urticae* larvae of two ages.

<table>
<thead>
<tr>
<th>Larvae age (h)</th>
<th>Concentration g AI/litre</th>
<th>Mean % mortality (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.0002</td>
<td>8.2 (± 6.14) a*</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>11.18 (± 6.21) ab</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>31.1 (± 17.77) b</td>
</tr>
<tr>
<td>18</td>
<td>0.0002</td>
<td>11.85 (± 13.61) a</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>22.33 (± 14.84) ab</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>75.58 (± 19.24) c</td>
</tr>
</tbody>
</table>

% Mortality data transformed to arcsin $\sqrt{\%}$. *Values with same letters indicate no significant difference ($P > 0.05$; Tukey's test [Zar 1984]).

ANOVA: concentration ($F = 19.7; df = 2,18; P = 0.05$); larva age ($F = 13.4; df = 1,18; P = 0.05$); concentration x larva age ($F = 3.6; df = 2,18; P = 0.05$).
Fig. 3. The response of *T. urticae* larvae of different ages to direct sprays of thuringiensin.

Larva 12 h
Larva 18 h
thuringiensin. According to Sebesta et al. (1981), arthropods are most susceptible to thuringiensin when high growth rates and physiological processes (such as metamorphosis) are occurring. Thuringiensin inhibits ribosomal DNA-dependent RNA polymerase; the level of ATP inhibition is influenced by the ratio of thuringiensin to ATP rather than their absolute concentrations (Sebesta et al. 1969). In poikilothermic organisms, a greater amount of ATP is formed when the temperature increases because the rate of metabolism, and particularly the rate of oxygen consumption, are affected (Rajagopal & Bursell 1965). Therefore, when more ATP is available, less competition for enzymatic binding sites occurs. At lower temperature, the increased susceptibility of larvae may be influenced by the ATP/thuringiensin ratio, and the significant interaction between temperature and thuringiensin concentration (Table 2) supports this contention. Further investigation of this mechanism could be pursued by determining the critical ratios of ATP and thuringiensin at different temperatures, and by studying the reversibility of the process in different developmental stages.

The persistence of thuringiensin activity against T. urticae larvae up to 8 d on bean leaves (Table 3) is consistent with the observations of other authors (Hall et al. 1971, Wolfenbarger et al. 1972, Neal et al. 1987, Mersie & Singh 1988, Royalty et al. 1990, 1991). The relatively long residual activity of thuringiensin, coupled with an increasing instantaneous mortality rate of mites exposed to thuringiensin residues (Chapter 2), suggests that significant control of field populations of mites should be achieved. However, further
assessement of the efficacy of thuringiensin against field populations while taking into account the phenology of each species (Robertson & Worner 1990) would be required to estimate probable field control with some degree of realism.

Host plant may also have a significant influence on field efficacy. Higher mortality occurred when T. urticae larvae were exposed to thuringiensin residues on peach and apple than on dwarf bean (Table 4). Many factors including leaf surface structure and physiology and the amount of chemical deposited could affect thuringiensin uptake and mortality (Wakou & Sugawara 1974, Asano & Kamei 1982, Marris & Chapman 1987). However no difference in mortality between the upper and lower leaf surfaces of all host plants tested was observed, despite the obvious differences in hair density on apple and bean leaves. Possibly other factors such as the chemical composition of leaf cuticles (Baker 1980), mite feeding behaviour and activity also may influence larval mortality on different leaf types. To clearly elucidate the importance of each factor, an improved method is required to test the responses of mites to miticides on different host plant surfaces such that factors such as droplet size, deposit density, leaf age and hair density are controlled. Studies also are necessary to evaluate the efficacy of thuringiensin under field conditions on a range of host plants for T. urticae and P. ulmi to be sure that an adequate level of control can be achieved.

Because penetration and absorption are the processes initially involved in pesticide translocation, thuringiensin would be expected to penetrate into cells through the aqueous
pathway due to its physio-chemical features (Singh & Mersi 1989). However, results in Table 5 suggest that sufficient thuringiensin does not penetrate into the leaf and, therefore, both translaminar and systemic activity were not detected by *T. urticae* larvae. Therefore, effective control of mite populations in the field will only occur when efficient spray coverage is achieved.

To enhance pesticide foliar uptake, surfactants have been widely used to decrease surface tension and increase coverage by spray droplets. The addition of an organosilicone surfactant did not significantly increase mortality of larvae that were exposed to thuringiensin residues (Table 6). This lack of enhanced effectiveness may indicate that the surfactant aided the penetration of thuringiensin into the leaf cuticle or epidermal cells and thereby reduced the potential for uptake by mites. A question remains about whether the translaminar or systemic action of thuringiensin would be improved by this surfactant, compared with the non-organosilicone surfactant used on an earlier experiment. When mites were directly sprayed with thuringiensin and surfactant, mortality increased (Table 6), however, this effect can be attributed to the intrinsic toxicity of the surfactant, i.e., no significant difference between thuringiensin with surfactant and surfactant alone occurred. Because surface retention could be a crucial feature of the contact toxicity (Ford & Salt 1987) of thuringiensin, further study of this aspect is warranted.

As previously stated, thuringiensin toxicity is greatest when higher growth rates and physiological processes occur and a
higher rate of RNA synthesis is required (Sebesta et al. 1981). In arthropods, the cellular content of DNA, RNA and proteins has been shown to steadily increase through larval development (Prudhomme & Couble 1979). The increasing susceptibility of *T. urticae* larvae with age (Figure 3) and the significant interaction between larval age and thuringiensin concentration on larval mortality (Table 8) is therefore likely to be related to the higher level of synthesis of nucleic acids that occur at the late larval stage. In contrast, a lower level of nucleic acid synthesis is likely to occur in an early larval stage, thereby making these younger larvae less susceptible. Such results may have considerable relevance to toxicological studies of chemicals that affect the metabolism that occurs during moulting. Ideally individuals should be tested when they are at their most susceptible stage of development because a maximum response to the chemical is expected. In addition, precision of LC estimates would be improved when test subjects are at the same stage of development.

In summary, results of this study indicated that the activity of thuringiensin against *T. urticae* larvae is affected by temperature, instar age and age of residues. Good early-season control of spider mites could be achieved because temperatures are generally lower and more immature mites are present, particularly with species like *P. ulmi*. Furthermore, the relatively long persistence of residues should allow the control of immature stages during early population development. However, further experiments with different *P. ulmi* and *T. urticae* stages are necessary to evaluate the influence of these
and other factors such as host age and effect of weather factors on residues persistence.
DURING THE DEVELOPMENT of certain pesticides, attention has been focused on their ability to inhibit the moulting process in arthropods. In particular, compounds like aminopterin, methotrexate, cyromazine, puromycin, cyclohexamine and several benzoylphenyl ureas have been reported to be efficient inhibitors of cuticle synthesis (Binnington & Retnakaran 1991). Although thuringiensiin, a β-exotoxin of Bacillus thuringiensis Berliner, was discovered in 1959 (McConnell & Richards, 1959) this product has not received the same attention as the aforementioned compounds. The research reported mostly relates to the biochemistry and toxicity of thuringiensiin (Sebesta et al. 1981, Beegle & Yamamoto, 1992). The available information on thuringiensiin indicates that it has high potential to inhibit
moulting in insects and mites, whereas with adults only fecundity and longevity are disrupted at high concentration (Sebesta et al. 1981, Royalty et al. 1990, 1991).

Thuringiensin belongs to a group of nucleic acid metabolism inhibitors. It acts by inhibiting ribosomal DNA-dependent RNA polymerase, one of the essential enzymes for transferring genetic information (Sebesta & Horská, 1968). It achieves this by out-competing ATP for enzymatic binding sites, due to its structural analogy with ATP (Sebesta et al. 1981). Toxicity is expressed when high growth rates and physiological processes (e.g., metamorphosis) are occurring in arthropods. These processes require higher rates of RNA synthesis than are necessary for the comparatively slower growth of adults (Sebesta et al. 1981).

In common with other chemicals known to affect nucleic acid metabolism, thuringiensin primarily disrupts epidermal activity because the epidermis is a major site of nucleic acid replication. Inhibition of nucleic acid metabolism could therefore affect cuticle formation and also the synthesis of enzymes, particularly chitin synthetase (Cohen 1987b).

Mothes-Wagner & Seitz (1981) and Mothes-Wagner (1986), when studying cuticle synthesis in *Tetranychus urticae* (Koch), found that the spider mite cuticle consists of a wax-containing epicuticle and a chitinous procuticle which reveals different layers when examined under high magnifications. The outer cement layer, which is very thin and often ruptured, overlies a very thin wax layer. This sequence is clearer in regions with cuticular glands which may secrete the wax layer. Between the
epicuticle and the endocuticle is the exocuticle which contains mostly granular material that extends to the lobes. The endocuticle shows a lamellation which is due to the helicoididal arrangement of chitin fibrils in a protein matrix. The cuticular ridges, which are formed from the epicuticle and parts of the exocuticle, also extend to the lobes (Mothes-Wagner, 1986). The deposition of epicuticle occurs on top of small hypodermal microvilli, whereas procuticle deposition occurs in the spaces between the epicuticle and hypodermal cell surfaces during the time when old cuticle persists. Hypodermal cells are densely packed with many rough endoplasmic reticulum (ER) cisterns, and free ribosomes (Mothes-Wagner & Seitz, 1981).

Because the effect of thuringiensin on the ultrastructure of spider mite cuticle has not been studied, two groups of experiments were performed. The first was designed to identify the structures in immature stages that were disrupted by the action of thuringiensin, while the second aimed to trace the process of cuticle formation and to explain the higher toxicity of thuringiensin to older larvae (Chapter 3). In both groups of experiments changes in the ultrastructure of the cuticle were determined by examining sequential sections of treated and untreated mites under an electron microscope.

**Methods and Materials**

**Sources of Mites.** All *T. urticae* used in this study were obtained from a colony that has been maintained by the Department of Entomology, Lincoln University, Canterbury since
1985 without exposure to pesticides. Mites were reared on French dwarf bean (*Phaseolus vulgaris*, cv. 'Tendergreen') in the laboratory at 21 ± 3°C; the photoperiod was 16:8(L:D).

**Treatment of Mites.** An experimental formulation of thuringiensin (ABG-6320), an aqueous suspension (5% [AI]) supplied by Abbott Laboratories, USA, was used in all experiments. The physical and chemical properties of thuringiensin have previously been reported by Sebesta et al. (1981). A Potter tower (Burkard Manufacturing Co. Ltd, Rickmansworth, U.K.) was used for applying thuringiensin suspensions in all experiments. Two ml of thuringiensin suspension was sprayed by the Potter tower on each occasion at 55 ± 5 kPa; a 10 s settling period was allowed. This resulted in a wet deposit of 1.25 ± 0.01 mg/cm².

For the first group of experiments whole French dwarf bean leaves placed on damp cotton wool in 85 mm diameter Petri dishes and were sprayed with three thuringiensin concentrations (0.005% g AI/l, 0.05% g AI/l, 0.5% g AI/l). A water-treated control was also established. Each treatment was replicated four times. After the residues had dried at least 25 larvae, protonymphs or deutonymphs were transferred to the leaves. Run-off from the leaves was prevented by confining mites in 12 mm diameter arenas surrounded by a sticky insect trap adhesive (Davis Gelatine N.Z. Ltd, Christchurch). Leaves were held at 23 ± 2°C and a photoperiod of 16:8(L:D) for ca. 30 h, after which mites were removed and fixed. Twenty five individuals were embedded per concentration for each stage.
The second group of experiments was designed to investigate the reason for different responses by young (2-h) and old (12-h) larvae (Chapter 3). To obtain sufficient numbers of larvae of uniform age, 200 females were allowed to lay eggs for 3-6 h on whole French dwarf bean leaves, placed on moist cotton wool in Petri dishes at 23 ± 2°C and a photoperiod of 16:8(L:D). Thirty larvae (0.5-h-old) were transferred into arenas and exposed to thuringiensin residues (0.005% g AI/l) for 6-30 h. Mites were then removed and fixed every 6 h to arrest the moulting process. In addition, thirty larvae of two ages (2- and 12 h) were exposed for 6 h on residues (0.005 g AI/l) and transferred to residues-free leaves to be fixed when both groups were 24 h old. A water-only control treatment was set up for comparison and, on each occasion, 25 larvae were embedded per treatment.

**Fixation.** The fixation technique used was based on that described by Rumpf (1990). Immature stages were placed on double-sided sellotape and punctured on the back or head with an entomological pin (000) to allow fixing and embedding chemicals to penetrate into the tissues. They were then fixed in 2.5% glutaraldehyde, buffered with 0.05M cacodylate at 4 ± 1°C overnight and washed in cacodylate buffer. The specimens were then postfixed in reduced 1% OsO₄ for 2 h at 4°C. This was followed by three separate washings in cacodylate and maleat buffer (30 min in each buffer). Specimens were then placed 0.5% uranylacetate-maleat-buffer and left overnight. The final step involved dehydration in graded ethanol (50-100%).
**Embedding.** To embed the specimens Spurr's technique (Spurr 1969), adapted by Rumpf (1990), was used. To achieve total replacement of the ethanol the following three-step infiltration process was used: i) constant rotation in 1:1 resin:ethanol mixture for 2 h; ii) constant rotation in 3:1 resin:ethanol mixture overnight; iii) constant rotation in pure resin for 8 h. All infiltration steps were carried out in a refrigerated room at 2°C. The specimens were then transferred to embedding capsules and covered with pure resin after being orientated under a binocular microscope. The resin was polymerized at 75°C for 24 h.

Approximately 100 serial cross sections per treatment of the opisthosomal cuticle of larvae, protonymphs and deutonymphs were obtained by cutting with a diamond knife on a LKB ultratome (Hayat 1970). The sections, 20-40 nm thick, were examined using a Zeiss 902 transmission microscope operated at 80 Kv. All sections were examined in an unstained condition because lead-stained sections (Reynolds 1963) were found to be inferior.

To get the best resolution in micrographs several combinations of exposure times, diaphragm aperture settings, magnifications and photographic papers were tested. An enlarger (De Vere 504) and an automatic print processor (Agfa Rapidoprint DD 1437) were used for all processing which was carried out in the photographic laboratory of Landcare Research New Zealand Ltd, Canterbury.
Results

Untreated Immature Stages. The fine structure of the intermoult cuticle of larvae at 6 and 12 h is shown in Plates 1 and 2. At these magnifications it is possible to observe ribosomes (R) and rough endoplasmic reticulum (rER) cisterns very close to the old cuticle (OC). At these ages, the old cuticle has not separated from the epidermis, the plasma membrane projections or microvilli (MV) are not visible and the new cuticulin layer has not been deposited. The procuticle (PC), lying immediately over the external surface of the epidermis, is distinguished from the epicuticle (EC) by its different electron density.

By 18 h (Plate 3) larvae have developed an extracellular space, usually called the exuvial space (ES), between the epidermis and the old cuticle. Deposition of the cuticulin (CU) layer of the second instar cuticle is evident in some areas. The cytoplasm contains rough endoplasmic reticulum and many free ribosomes (R). The electron dense appearance is due to the close packing of free ribosomes in the cell. Ecdysial droplets are not visible within the exuvial space, however, coated vesicles (CO) with ecdysial droplets are located at the apical border of the epidermis.

At about 24 h, deposition of the new epicuticle layer is conspicuous throughout the section (Plate 4). Nuclei (N) of the epidermal cells are relatively large and irregular and contain one or more nucleoli (NI). In Plate 5, it is possible to
Plate 1. Transverse section of the larva cuticle 6 h after hatching, showing the entire old cuticle (OC), epicuticle (EC), procuticle (PC), rough endoplasmic reticulum (rER), ribosomes (R), and mitochondria (M). X 87,000

Plate 2. Transverse section of the larva cuticle 12 h after hatching, showing the entire old cuticle (OC), epicuticle (EC), procuticle (PC), rough endoplasmic reticulum (rER), ribosomes (R), and mitochondria (M). X 97,000
Plate 3. Transverse section of the larva cuticle 18 h after hatching, showing the entire old cuticle (OC), exuvial space (ES), new cuticle (NC), microvilli (MV), cuticulin (CU), ribosome (R), rough endoplasmic reticulum (rER), and coated vesicle (CO). X 40,000

Plate 4. Transverse section of the larva cuticle 24 h after hatching, showing old cuticle (OC), exuvial space (ES), new cuticle (NC), microvilli (MV), cuticulin (CU), ribosome (R), rough endoplasmic reticulum (rER), nuclei (N), and nucleoli (NI). X 12,000
Plate 5. Transverse section of the larva cuticle 24 h after hatching, showing old cuticle (OC), new cuticle (NC), microvilli (MV), cuticulin (CU), ribosome (R), dense layer (DL). X 115,000

Plate 6. Transverse section of the larva cuticle 30 h after hatching, showing old cuticle (OC), new cuticle (NC), exuvial space (ES), microvilli (MV), cuticulin (CU), dense layer (DL). X 40,000
observe that cuticulin of the new cuticle (NC) appears in a narrow electron-dense line over the surface of the microvilli. Beneath the cuticulin an electron dense region similar to the inner region of the cuticulin layer of the old cuticle occurs. This suggests that part of the dense layer (DL) may be secreted concurrently with the cuticulin, rather than sequential deposition of cuticulin followed by the dense layer (Filshie, 1970).

After 30 h (Plate 6), further secretion of cuticulin causes lateral extension and fusion of neighbouring areas until the membrane is complete. The dense layer is, at this time, in an advanced stage of development. From 30 h (Plate 7) till ecdysis, the cuticle continues to increase in thickness, and the old endocuticle becomes separated from the new epicuticle. The formation of the epicuticle will be completed and the new procuticle will be deposited beneath the epicuticle.

With protonymphs and deutonymphs (Plates 8 and 9), after 24 h the extracellular space has developed between the old and the new cuticle, as was observed in larvae. At this time deposition of the new cuticulin layer is evident. Cuticulin appears in electron dense areas over the surface of the microvilli. At 24 h protonymphs and deutonymphs show structures similar to those found in larvae.

**Treated Immature Stages.** Plate 3 showed that 18-h-old larvae have started cuticle formation and secretion of the new cuticle is at the first phase. However when immature stages were exposed to thuringiensin residues on French dwarf bean
Plate 7. Transverse section of the larva cuticle 36 h after hatching, showing old cuticle (OC), new cuticle (NC), exuvial space (ES), epicuticle (EC) with dense layer (DL), and procuticle (PC). X 67,000

Plate 8. Transverse section of the protonymph cuticle 24 h after emerging, showing old cuticle (OC), new cuticle (NC), exuvial space (ES), epicuticle (EP), microvilli (MV), rough endoplasmic reticulum (rER), mitochondria (M), and nucleus (N). X 40,000
Plate 9. Transverse section of the deutonymph cuticle 24 h after emerging, showing old cuticle (OC), new cuticle (NC), exuvial space (ES), cuticulin (CU), rough endoplasmic reticulum (rER), and mitochondria (M). X 24,000

Plate 10. Transverse section of the treated larva cuticle 18 h after hatching, showing no cuticulin synthesis, old cuticle (OC), rough endoplasmic reticulum (rER), mitochondria (M), and nucleus (N). X 26,000
leaves the cuticle formation process was disrupted. This effect on cuticle formation only is perceivable in treated larva after 18 h, when no cuticulin envelope was observed (Plate 10).

At 24 and 30 h, the inhibition of cuticle synthesis is more evident, microvilli are not visible, mitochondria (M), endoplasmic reticulum and ribosomes appear abnormal in shape (Plates 11 and 12). Therefore, in treated larvae, the cuticulin (or outer epicuticle) and its component lipids and proteins were not synthesized, and subsequently the inner epicuticle or dense protein layer and procuticle also were not formed.

The effects of thuringiensin on protonymphs and deutonymphs were similar to those for larvae. Plates 13 and 14 show protonymph and deutonymph stages that were exposed to residues for 24 h and where cuticle formation was disrupted and epicuticle was only partially deposited. In the water-only control treatment, the cuticulin envelope was formed as the first layer of new cuticle, where it arises from the tips of the plasma membrane plaques and defines the outer limit of the cuticle (Plates 8 and 9).

Two-h-old larva exposed to residues for 6 hours were not affected by thuringiensin and the synthesis of cuticle proceeded normally (Plate 15). However, 12-h-old larvae that remained for the same time on thuringiensin residues were were unable to form new cuticular material (Plate 16).
Plate 11. Transverse section of the treated larva cuticle 24 h after hatching, showing no cuticulin synthesis, old cuticle (OC), rough endoplasmic reticulum (rER), mitochondria (M), and ribosome (R). X 83,000

Plate 12. Transverse section of the treated larva cuticle 30 h after hatching, showing no cuticulin synthesis, old cuticle (OC), mitochondria (M), and ribosome (R). X 83,000
Plate 13. Transverse section of the treated protonymph cuticle 24 h after emerging, showing disrupted cuticulin synthesis (DC), old cuticle (OC), mitochondria (M), rough endoplasmic reticulum (rER), and nucleus (N). X 20,000

Plate 14. Transverse section of the treated deutonymph cuticle 24 h after emerging, showing disrupted epicuticle synthesis (EP), and old cuticle (OC). X 83,000
Plate 15. Transverse section of the 2-h-old larva cuticle and exposed to thuringiensin residues for 6 h, 24 h after hatching, showing old cuticle (OC), new cuticle (NC), exuvial space (ES), new cuticle (NC), ribosome (R), rough endoplasmic reticulum (rER), and nucleus (N). X 24,000

Plate 16. Transverse section of the 12-h-old larva cuticle and exposed to thuringiensin residues for 6 h, 24 h after hatching, showing no cuticulin synthesis, and old cuticle (OC). X 99,000
Discussion

The principal objective for studying the effects of thuringiensin on the ultrastructure of TSM was to determine how and when it inhibited cuticle formation. By understanding these aspects, more efficient use of thuringiensin may result.

Because the effects of thuringiensin on cuticle formation in spider mites have not been reported, an experimental approach for larvae was developed. T. urticae larvae were chosen for these experiments because they were shown to be the stage most susceptible to thuringiensin. Other benefits of using larvae included uniformity of age and short developmental period. On the negative side, their small size and lack of information on cuticle structure were problems. The success of this study also depended on using an appropriate thuringiensin concentration and exposure period to disrupt moulting without killing mites at any time during their development. This was achieved by trial and error.

The external manifestation of moult disruption was similar to those observed with other cuticle inhibitors, i.e., juvenile individuals were unable to reach the succeeding chrysalid stage. However the biochemical processes involved and the structures disrupted differ between inhibitors (Fogal and Fraenkel 1969, Oberlander et al. 1980, Chen and Riddiford, 1981; Sebesta et al. 1981, Mothes-Wagner and Seitz 1982, Hajjar 1985, Retnakaran et al. 1985, Cohen 1987a, b). From the observations made in this study the synthesis of cuticulin was consistently disrupted and consequently no epicuticle was formed in T. urticae immature
stages when they were exposed to thuringiensin. In contrast nikkomycin, another inhibitor of cuticle synthesis, has been shown to affect chitin synthesis of the procuticle of *T. urticae* (Mothes-Wagner & Seitz 1982). Because the results of this study showed that thuringiensin mainly affected synthesis of epicuticle, a non-chitinous cuticle layer that is composed of lipids and proteins (Neville 1975), they support the contention that thuringiensin is an efficient inhibitor of the moulting process at the nucleic acid level by blocking ribosomal-protein synthesis (Cohen 1987b, Sebesta et al. 1981). Furthermore, it may be assumed that thuringiensin blocks the formation of new cuticle earlier than other cuticle inhibitors, because cuticulin is the first layer formed of the new cuticle (Binnington and Retnakaran 1991).

Cuticle formation was seen to be disrupted gradually and larvae were affected from 12 h onwards. Thus, when 12-h-old larvae remained on residues for 6 h they were unable to synthesize cuticular material. Because larvae less than 12-h-old were not affected, it may be inferred that the higher susceptibility to thuringiensin may be related to the low rate of ATP metabolism (Sebesta & Horská, 1968) during the early stages of development.

Although protonymphs and deutonymphs required higher thuringiensin concentrations to cause similar moulting disruption effects, metabolic reasons could explain such a response. According to Sebesta et al. (1981), thuringiensin achieves its effect by out-competing ATP for enzymatic binding sites, and the inhibition becomes reversible depending on the
ratio of ATP to thuringiensin (Sebesta et al. 1969). Therefore, it could be hypothesized that protonymphs and deutonymphs have higher amounts of ATP and consequently a higher amount of thuringiensin would be necessary to out-compete ATP for enzymatic binding sites.

This study has clearly shown that it is possible to identify the effect of thuringiensin at the ultrastructural level by comparing treated and untreated immature T. urticae. The study has also shown that the time of exposure to thuringiensin is critical and that the higher susceptibility of older larvae is related to the stage of cuticle development. The results of this study also suggest some important practical consequences for toxicological studies, particularly with respect to appropriate times to expose mites to moult inhibitors like thuringiensin.

Further ultrastructural studies are necessary to determine the exact time that epicuticle synthesis in protonymphs and deutonymphs is disrupted. Also it is suggested that it may be important to study the reversibility of the inhibition of ribosomal DNA-dependant RNA polymerase by thuringiensin and its consequences for spider mite development.
Chapter 5

General Discussion

Introduction

MOST RESEARCH reported to date on thuringiensin has been related to its biochemical features and mode of action (Sebesta et al. 1981). With insects thuringiensin has been shown to be toxic to immature stages (Heimpel 1967, Bond et al. 1971) and with spider mites and insects it has been reported to affect development and fecundity (Krieg 1968, Hall et al. 1971, Ignoffo & Gregory 1972, Perring & Farrar 1986; Neal et al. 1987; Royalty & Perring 1987; Royalty et al. 1990, 1991). These studies also suggest that thuringiensin exerts its primary action through contact exposure and it is likely that oral uptake is of minor importance because thuringiensin has little or no systemic or translaminar action in plants (Neal et al. 1987).

Although aspects of thuringiensin's biochemical mode of action are well understood (Sebesta et al. 1981) little is known about the effects of thuringiensin on spider mite cuticle development. An understanding of this aspect is essential to
explain the observed effects of thuringiensin on spider mites. Therefore the main objectives of this study were:

a) to develop an appropriate bioassay method to study the effects of thuringiensin on spider mites;

b) to determine the miticidal activity of thuringiensin with both *T. urticae* and *P. ulmi*, by investigating the responses of each developmental stage and comparing their relative susceptibilities to thuringiensin through direct or residual exposure, and by determining the effects of thuringiensin on *T. urticae* reproduction and population development;

c) to investigate the influence of different factors (temperature, host plant, active ingredient mobility, surfactant, residue age and larval age) on thuringiensin toxicity;

d) to investigate the effect of thuringiensin on the development of *T. urticae* cuticle.

The following discussion considers each of these main objectives in order and then integrates the information available on thuringiensin to discuss a range of practical implications.

**Discussion**

**Bioassay Methods.** Because the selection and use of appropriate bioassay methods are fundamental to pesticide research (Dennehy et al. 1983, Kabir 1992) special attention needs to be given to this aspect. Ideally bioassay methods must be simple, provide reproducible results and, for some applications, simulate the conditions of field treatment as
closely possible (Walker et al. 1973). However, no single method can always be used because each has particular advantages and disadvantages and often serve different aims. Therefore the type and mode of action of the pesticide, the species being tested and the aims of the specific research must be taken into account when selecting a bioassay method.

The substrate used for containing mites in a bioassay was considered to be an important factor, because earlier studies had suggested that long observation periods were required due to thuringiensin’s slow-acting nature (Royalty & Perring 1987; Royalty et al. 1990, 1991). As consequence, whole excised leaves were used in most bioassays as they did not deteriorate to the same extent as leaf discs. However this factor did not prove to be a limitation when evaluating the toxicity of thuringiensin to individual mite stages because actual mortality occurred at the succeeding stage within 24-48 h exposure. When it was necessary to study other prolonged effects of thuringiensin the whole leaf provided an adequate food supply.

When selecting a suitable bioassay method, the nature and mode of thuringiensin’s action was also a priority consideration. Preliminary tests gave very variable results and strongly suggested that the susceptibility of juvenile mites was age-related. It was reasoned that by confining the age of mites used in bioassays to a narrow range higher precision of LC estimates (shorter 95% CIs, Robertson et al. 1984) would be achieved. Therefore, immature mites selected for testing were always approximately half-way through any developmental stage. It was easier to select this developmental stage for larvae than other immature stages and this was generally reflected by the shorter 95% CIs achieved for LC values in the concentration-
mortality bioassays. This factor, along with the mortality criterion adopted in this study (failure to enter the next developmental stage), were main the differences between the bioassay methods used in this study and others, e.g., Royalty et al. (1990, 1991). These investigators also appear not to have considered the age-related responses of mites to thuringiensin. It would be desirable in the future to consider the design and optimal total sample size required to enhance the precision of LC estimates. The experimental approach used by Kabir (1992) for testing propargite against spider mites could be adapted for thuringiensin.

**Thuringiensin Toxicity.** The significant differences shown between the slopes and LC values for direct and residual exposure for different instars and the increasing LC ratios for immature stages between direct and residual exposure with developmental stage (Chapter 2), indicate that spider mites are most susceptible when larvae are directly sprayed with thuringiensin. In contrast adult mites are very tolerant to thuringiensin. Observation of treated immature mites showed that the effects of thuringiensin toxicity were similar to those of clofentezine and hexythiazox, i.e., failure to emerge in the succeeding stage (Aveyard et al. 1986, Chapman & Penman 1988), although thuringiensin has no ovicidal activity. In practice, the lack of any appreciable adulticidal activity of thuringiensin may need to be offset by the incorporation of an adulticidal contact miticide in a spray programme to prevent feeding damage. Although direct spraying of mites was always more effective than when mites were exposed to thuringiensin residues, this difference may not be reflected under field
conditions because 100% spray coverage is not possible and immature mites are small targets for pesticide droplets (Muthali & Wyatt 1986). These investigators found that less than 5% of spray droplets of 100 μm diameter directly hit eggs of T. urticae. It is likely that residues on leaves will therefore be the main route of exposure and that the activity of spider mites may predispose them to picking up greater quantities of thuringiensin. Spray droplet, formulation and leaf surface characteristics will also influence the pick-up of miticide by mites (Wakou, & Sugawara 1974, Munthali & Scopes 1982, Munthali & Wyatt 1986).

Royalty et al. (1990, 1991) did not find any significant difference between the responses of immature stages of T. urticae or P. ulmi but found significant differences between the susceptibility of immatures and adults. It is likely that the different results obtained in this study are due to the differences in bioassay methods employed. Furthermore, in the Royalty et al. tests the age of immature stages was not controlled and, therefore, the LC estimates did not reflect the differences between the most susceptible individuals of each instar to thuringiensin. The significance of this point was reinforced when the responses of three different age groups were investigated (Chapter 3).

The effectiveness of thuringiensin varied markedly between P. ulmi and T. urticae. LC₅₀ and LC₉₀ₙs for each P. ulmi instar were significantly lower than the corresponding values for T. urticae for direct exposure. These differences probably reflect a different sensitivity of DNA-dependent RNA polymerase (Beebee & Bond 1973 a,b). In addition, varying degrees of susceptibility to thuringiensin may be due to a different ratio
of thuringiensin to ATP at specific developmental stages among species (Sebesta 1969). However, other factors such as mite behaviour, poison penetration, physiological and biochemical differences could also account for susceptibility differences between the two species (Robertson & Priesler 1992, Holland 1992). Interspecific differences in susceptibility have been noted in other miticide studies (Ifter & Hall 1983, Gough & Qayyom 1987, Knowles et al. 1988, Knight et al. 1990, Holland 1992). It also likely, that previous exposure to miticide and inherent strain variation may also be responsible for differences in responses. However the differences in the response to thuringiensin between immature *T. urticae* and *P. ulmi* have not been previously reported, and it may suggest that the field application rates of thuringiensin could be lower where *P. ulmi* was the dominant spider mite species on a crop.

**Population Mortality.** The 100% inhibition of the development of motile stages achieved in one *T. urticae* generation shows the potential of thuringiensin residues to control a mite population. The evaluation made on one generation suggests that the efficacy of thuringiensin could be underestimated if it were evaluated only on single stage, since the accumulated uptake and effects on mites are ignored. As indicated by Robertson & Worner (1990), population toxicology is far more realistic than prediction based on the response of any one stage. These results show that a significant reduction of the mite population occurred beyond the stage that was initially exposed to the chemical. Therefore, it is possible that good early-season control of spider mites could be achieved because more immature mites are present, particularly with species like
Because the field rate is likely to be higher than the concentration levels tested here and that thuringiensin residues have a relatively long period of activity, the potential for suppression of mite field populations is predicted to be high. Furthermore, the prolonged residual activity of thuringiensin would probably offset the lack of ovicidal effects because when larvae hatch they would be exposed immediately to a lethal concentration. Further observations are needed on more than one generation under semi-field and field conditions to evaluate the effects of thuringiensin residues where mites are not constantly exposed to residues.

**Fecundity.** Exposure of *T. urticae* to residues or direct sprays significantly affected fecundity, which was inhibited by > 90% after 4 d (Chapter 2). The maximum reduction of fecundity for *T. urticae* recorded by other workers was 25% (Royalty et al. 1990) and, again, it is likely that different bioassay methods, formulations and mite strains used to evaluate fecundity could account for the differences. The main methodological difference was that Royalty et al. (1990) determined the fecundity of the F₁ generation exposed to residues instead evaluating the effect on recently-mated females exposed to residues. However, the principal mode of action of thuringiensin probably could explain the observed reductions in fecundity, because thuringiensin would inhibit DNA-dependent RNA polymerase (Sebesta et al. 1981) and subsequently block mitosis during egg development in the ovary.

Reductions of fecundity have similarly been found for *T. urticae* treated with tetradifon (Bæth & Davidson 1959), cycloprate (Asano & Kamei 1977), fenpropathrin (Rasmy & Elsawy...
1988) clofentezine (Chapman & Marris 1986) and fluvalinate (Holland 1992). Different reasons have been given to explain these reductions, e.g., reduced feeding caused lower fecundity in mites (Kasamatsu & Fujita 1986, Iftner et al. 1986) or reduced oviposition due to chronic poisoning from contact with the residues. Considering that fecundity is the second most important factor influencing the intrinsic rate of increase (Snell 1978), the reduction of fecundity by thuringiensin may exert an important effect on population development and might compensate for low toxicity to adults. However, further studies are necessary to fully exploit the fecundity inhibition effect on spider mite populations (e.g., different species, surviving females from treated deutonymphs).

Factors that influence thuringiensin toxicity. The previous discussion has shown that both the dose-time relationship, route of exposure and species influence the responses of spider mites to thuringiensin. In addition there are other factors that modify the responses of mites to thuringiensin. This study showed that thuringiensin toxicity mainly was influenced by temperature, instar age, age of residues and host plant (Chapter 3).

Temperature is one of the most important factors influencing the biology and response of arthropods to pesticides. The developmental rate has been shown to increase with temperature for a range of spider mite species (Tanigoshi et al. 1975; Herbert 1981, Perring et al. 1984). Miticides such as cyhexatin, dicofol, chlorobenzilate and propargite are all reported to have a positive temperature-toxicity correlation (Fisher & Hansell 1964; Stenseth 1976; Everson & Tonks 1981;
James et al. 1988). In contrast, the toxicity of only a few miticides have been reported to be negatively correlated with temperature, e.g., dicofol and tetradoxon (Hassan et al. 1970), and their mechanisms of action in relation to temperature have not been explained. However with insecticides, including pyrethroids, it has been assumed that a greater rate of penetration occurs through the insect cuticle at higher temperature (Blum & Kearns 1956). Holland (1992) suggested that a positive temperature correlation may be related to an increase in activity that increases contact with the pesticide. The negative temperature-toxicity relationship for thuringiensin with immature stages can probably be explained by the fundamental mode of action of thuringiensin. At lower temperatures less ATP is formed, because the rate of metabolism and particularly the rate of oxygen consumption are affected (Rajagopal & Bursell 1966). Consequently there would be less ATP available and more competition with thuringiensin for enzymatic binding sites would occur. Further investigation of this mechanism could be done by determining the critical ratios of ATP and thuringiensin at different temperatures and by studying the reversibility of the process in different developmental stages. It may also be important to determine whether thuringiensin uptake by spider mites at different temperatures is also affected.

The long persistence of a pesticide is usually related to its chemical stability thus giving it long life in soil, plant and animal tissues. It also indicates that they are not readily broken down by ultraviolet light, heat, microorganisms and enzymes (Ware 1983). Thuringiensin is reported to be a relatively persistent active ingredient (Sebesta et al. 1981)
and it remained effective against *T. urticae* for 8 d (Chapter 3). It is also not readily absorbed and transformed by plant tissue and this probably explains its long activity against *T. urticae*, which is consistent with the observations of other authors (Hall et al. 1971, Wolfenbarger et al. 1972, Neal et al. 1987, Mersie & Singh 1988, Royalty et al. 1990, 1991). Because laboratory conditions differ so markedly from the field, the persistence and activity of thuringiensin residues should be tested under field conditions where all the biotic and abiotic factors are present.

Higher mortality occurred when *T. urticae* larvae were exposed to thuringiensin residues on peach and apple than on dwarf bean indicating that the host plant is a significant factor influencing pesticide efficacy (Chapter 3). Many factors including leaf surface structure and physiology and the amount of chemical deposited could affect the uptake of thuringiensin by both the plant and mites and therefore influence mite mortality (Wakou & Sugawara 1974, Asano & Kamei 1982, Marris and Chapman 1987). Translaminar and systemic actions of thuringiensin were not detected by *T. urticae* larvae, as it is likely that sufficient thuringiensin did not penetrate into the leaf. This strongly suggests that contact toxicity would the main cause of mite mortality.

Addition of surfactant (Silwett L-77) did not affect thuringiensin toxicity, and no explanation of this can be gained from this study. Addition of surfactant might affect thuringiensin availability on leaf surfaces by either reducing or enhancing the potential for uptake by mites (Ford & Salt 1987). Retention of thuringiensin on leaf surfaces would be crucial as contact exposure appears to be most important.
Characteristics of the formulation used would also influence the contact and pick-up of thuringiensin by mites. This problem requires further careful study to determine the importance of oral and contact uptake of thuringiensin. It may also help account for the report that thuringiensin is much less toxic orally than by direct routes of administration in insects (Sebesta et al. 1969).

The increasing susceptibility of \textit{T. urticae} larvae with age is likely to be related to the higher level of nucleic acid synthesis which occur at the late larval stage (Sebesta et al. 1981). In contrast, a lower level of nucleic acid synthesis is likely to occur at an early larval stage, thereby making them less susceptible (Prudhomme & Couble 1979). This explanation may have considerable relevance to toxicological studies involving chemicals that affect moult metabolism. Ideally, individuals should be tested when they are at their most susceptible stage of development and, therefore, the precision of LC estimates also would be improved when test subjects are physiologically homogeneous. The results from the study on the responses of three \textit{T. urticae} larval ages confirm that the precision of LC estimates would be higher if the most susceptible larval age (18 h) was tested. Furthermore, the precision of LC estimates could explain the significantly different responses between immature stages achieved in this study, compared to the Royalty et al. (1990, 1991) studies where no significant differences between immature stages were detected. These observations could be extended to other so-called ‘growth regulators’ because, like thuringiensin, they affect specific metabolic processes which are synchronised to specific developmental times. Therefore, with bioassays of growth
regulators, there is a primary need to identify the most susceptible age to expose before conducting experiments to estimate LCs values and other toxicological parameters.

**Ultrastructure.** Detailed investigation of the ultrastructure of *T. urticae* cuticle confirmed that thuringiensin can disrupt the synthesis of larval cuticle after 6 h exposure, and protonymph and deutonymph cuticle synthesis after 24 h exposure. These observations contradict the assertion of other investigators that thuringiensin is a slow-acting active ingredient (Krieg 1968; Hall et al. 1971; Perring & Farrar 1986; Neal et al. 1987; Royalty & Perring 1987; Royalty et al. 1990, 1991) and therefore this claim should be abandoned. Observations on the cuticle ultrastructure made in this study help to distinguish between the internal disruption to developmental processes occurring in the mites and the outward manifestation of poisoning that apparently are similar to 'growth regulators'. The results of this study showed that thuringiensin mainly affected the synthesis of epicuticle, a non-chitinous cuticle layer that is composed of lipids and proteins (Neville 1975). They also support the contention that thuringiensin is an efficient inhibitor of the moulting process at the nucleic acid level by blocking ribosomal-protein synthesis (Cohen 1987b, Sebesta et al. 1981). Furthermore, it may be assumed that thuringiensin blocks the formation of new cuticle earlier than other cuticle inhibitors, because cuticulin is the first layer formed of the new cuticle (Binnington & Retnakaran 1991). In contrast nikkomycin, another inhibitor of cuticle synthesis, has been shown to affect chitin synthesis of the procuticle of *T. urticae* (Mothes-Wagner & Seitz 1982). It
raises a question as to whether the moult-disruption effects of other miticides should also be investigated more closely, e.g., hexythiazox (an ovo-larvicide). This may be valuable because it could be suggest the appropriate 'windows' to target miticide applications in bioassays. Another potential use of this approach would be determine whether there was any similarity in the effects between thuringiensin and the ovo-larvicides to which some spider mite species have developed resistance (e.g., hexythiazox, clofentezine) (Edge et al. 1987).

However this first ultrastructural study approach using Turticae larva has been very appropriate to the aims proposed. Improvements to some of the techniques used are needed, especially the fixation of larvae. Because of the small size of larvae, efficient penetration of chemicals into the tissues was quite difficult to achieve. Further ultrastructural studies are also needed with protonymphs and deutonymphs to fully determine the effects of thuringiensin on cuticle synthesis on these stages and to define the susceptibility 'windows'.

Use of Thuringiensin in Spider Mite Control Programmes. A new miticide represents an important pest management tool, more so these days because resistance and registration difficulties have greatly reduced the number of commercially available products. Cyhexatin, dicofol and chlordimeform are examples of miticides whose registrations have been cancelled or subjected to toxicological re-evaluations (Royalty et al. 1991).

Thuringiensin has an intrinsic mode of action which is toxic to all animals (Laird et al. 1990), therefore its effects on several potential target organisms should be studied. It has been confirmed that thuringiensin is toxic to some beneficial
organism such as *Metaseiulus occidentalis* (Nesbitt) (Hoy & Ouyang 1987), *Neoseiulus fallacis* (Laird et al. 1990) and bees (Cantwell et al. 1964). It has also been confirmed that, after 7 months of selection pressure in the laboratory, resistance in a *T. urticae* mite population was achieved (Dennehy & Lueloff 1992). Due to its mode of action the likelihood of cross-resistance to thuringiensin has been assumed to be lower than for nerve poisons currently used for spider mite control (Royalty et al. 1990, 1991), however, it does not entirely preclude the chances of cross-resistance occurring. For example, many spider mite species have develop resistance to organotin miticides, and the mode of action of organotins is by inhibition of oxidative phosphorylation by blocking the formation of ATP (Ware 1983). This biochemical path might be considered as a basis for cross-resistance with thuringiensin since both are involved with ATP metabolism. Closer study of these metabolic relationships between toxicants and ATP would therefore be useful.

Notwithstanding the previous comments, it will be necessary develop a miticide resistance management programme to ensure the preservation of mite susceptibility to thuringiensin. This could involve tactics which reduce the number of applications, lower the application rates, allow untreated refugia for mites, and avoid the use of persistent formulations that affect only one life cycle stage (Chapman 1990). Therefore, any reduction of selection pressure on a spider mite population will minimise the risk of resistance and contribute to a long commercial life of thuringiensin. As an example, clofentezine and hexythiazox have been restricted to one application on tree fruits in New Zealand (Prince et al. 1989) and still remain effective in many
orchards. Repeated use of these miticides in Australia resulted in resistance development in one or two seasons (Edge et al. 1987).

However, it is essential to design a rational strategy to incorporate thuringiensin into IMC programmes to minimise the risk of resistance development and the toxic effects to beneficial organism. This could be achieved by using mite phenology to determine spray timing and ecological 'windows' when natural enemies are not present (Croft & Hoyt 1983). According to the results of this study, thuringiensin could be successfully used to control early-season P. ulmi populations where lower temperatures would also favour its efficacy. Furthermore, it may be possible to use lower rates of thuringiensin to re-adjust the predator-prey ratio in favour of predators (Hoyt 1969, Croft 1972, Croft & McGroarty 1977), and to incorporate an adulticidal miticide to prevent adult feeding damage. Forrester (1993) has further suggested the possibility of using thuringiensin to complement the activity of Bacillus thuringiensis Berliner, and a mixture of the two could prove to be quite useful in integrated pest management and resistance management programmes. Although thuringiensin has been suggested as being chemically-safe to humans due its rapid enzymatic degradation in mammals (Sebesta et al. 1981), early season application(s) could be used to ensure the absence of residues at harvest.
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