TOXICITY OF THURINGIENSIN ON IMMATURE AND ADULT STAGES OF *Tetranychus urticae* KOCH AND *Panonychus ulmi* (KOCH) (ACARINA: TETRANYCHIDAE)[1]

Toxicidad de thuringiensin en estados inmaduros y adultos de *Tetranychus urticae* Koch y *Panonychus ulmi* (Koch) (Acarina: Tetranychidae)

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ABSTRACT

The direct and residual toxicity of thuringiensin on different stages of *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch), 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 days. Complete suppression of *T. urticae* population development was achieved when the F_1 generation was exposed to thuringiensin residues, however, the level of suppression was concentration dependent. The results suggest that thuringiensin acts in a relatively short time. Therefore, the high toxicity of thuringiensin on immature spider mite stages and the sublethal effects on females suggest that thuringiensin may successfully control field populations. The use of thuringiensin in spider mite control programmes is discussed. Practical suggestions on the development of bioassays for active ingredients of this type are also discussed.

Key words: β-exotoxin, miticide, direct toxicity, residual toxicity, bioassay.

RESUMEN

La toxicidad directa y residual de thuringiensin sobre diferentes estados de *Tetranychus urticae* Koch y *Panonychus ulmi* (Koch), e influencia sobre la reproducción y desarrollo de la población de *T. urticae* fueron evaluadas en bioensayos de laboratorio. La toxicidad directa fue más alta que la toxicidad residual sobre todos los estados de *T. urticae*. Los estados inmaduros de *T. urticae* y *P. ulmi* fueron más susceptibles a thuringiensin que el estado adulto. *P. ulmi* fue más susceptible a thuringiensin que *T. urticae*. La fecundidad de *T. urticae* fue significativamente reducida en las hembras expuestas a residuos durante 2 días. La completa supresión en el desarrollo de la población de *T. urticae* fue lograda cuando la generación F_1 fue expuesta a residuos de thuringiensin, la cual dependió de la concentración utilizada. La alta toxicidad de thuringiensin sobre estados inmaduros

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de arañitas y el efecto subletal en hembras sugieren que thuringiensin puede controlar exitosamente poblaciones de campo. Además se discute el empleo de thuringiensin en programas de control de arañitas y el desarrollo de bioensayos.

Palabras clave: β-exotoxina, acaricida, toxicidad directa, toxicidad residual, bioensayos.

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 the European red mite *Panonychus ulmi* (Koch), and the twospotted spider mite, *Tetranychus urticae* Koch. Chemical control of both mites is commonly achieved with a narrow range of acaricides. However, the use of conventional acaricides has been severely restricted by resistance, intolerable residues on export products, selectivity to beneficial species, toxicological and environmental problems. To overcome these problems, the search for alternatives to conventional pesticides has intensified over the last two decades (Roush and 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, dialysable 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, also known as β-exotoxin, fly factor, heat-stable toxin, thermostable toxin, and thuringiensin, were discovered by McConnell and Richards (1959). Studies by Mohd-Salleh *et al.* (1980) indicated the existence of more than one type of heat-tolerant exotoxin. The existence of a second heat-tolerant exotoxin was chemically confirmed by Levinson *et al.* (1990), who named it type II β-exotoxin, which was more specific than the type I β-exotoxin, and was specially active against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say).

Type I β-exotoxin, thuringiensin, acts by inhibiting ribosomal DNA-dependent RNA polymerase and competing 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 also have contact toxicity and oral toxicity to arthropods (Grau, 1986; Hoy and Ouyang, 1987).

Krieg (1968) proved the effectiveness of β-exotoxin on active stages of *T. urticae*, whereas Hall *et al.* (1971) found that β-exotoxin was highly toxic to adult and immature citrus red mites, *Panonychus citri* (McGregor). Grau (1986) showed that a pure preparation of β-exotoxin (A.B.G. 6162) was toxic to *T. urticae* and pacificus spider mites *Tetranychus pacificus* (McGregor). However, Perring and Farrar (1986) observed no control of *T. urticae* on melons. Hoy and Ouyang (1987) found that β-exotoxin was toxic to adult females of *T. pacificus* and *Metaseiulus occidentalis* (Nesbitt) within 48-96 h of when they were treated at rates of 0.125-4 times the proposed field rate (0.0528 g AI L^{-1}) (AI:active ingredient).

Later, Royalty *et al.* (1990, 1991) discovered that the toxicity of two formulations of thuringiensin to *T. urticae* and *P. ulmi* was not significantly different for protonymphs and deutonymphs. However, both of 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 on 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 objectives of this study were to compare the direct and residual toxicity of thuringiensin on immature and adult stages of *T. urticae* and *P. ulmi* and to determine the effects of thuringiensin on *T. urticae* reproduction and population development.

MATERIALS AND METHODS

This study was done during 1991-1993 at Lincoln University, Canterbury, New Zealand. 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 laboratory rearing difficulties.

Sources of mites. The Department of Entomology and Animal Ecology, Lincoln University, Canterbury, New Zealand has maintained a *T. urticae* strain unexposed to pesticides since 1985. Mites of this strain were reared on French dwarf beans (*Phaseolus vulgaris* L., cv. 'Tendergreen') in the laboratory under a 16:8 (L:D) photoperiod. The temperature and humidity were not controlled but were approximately 21 ± 3°C and 60 ± 15% RH. 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 peaches (*Prunus persica* S. and Z., 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, 5% AI, aqueous suspension, Abbott Laboratories, North Chicago, Illinois, 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, UK) was used for applying thuringiensin in all experiments. On each occasion 2 mL of thuringiensin suspension were sprayed at 55 ± 5 kPa; followed by a 10 s settling period. This technique resulted in a wet deposit of 1.25 ± 0.01 mg cm^{-2} (ten 22 mm diameter glass microscope slide cover-slips were weighed before and after spraying to obtain the mean wet deposit).

For a concentration-mortality response to be estimated using 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 to be approximately midway 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 leaflets were placed upside down on moistened cotton in an 85 mm diameter Petri dish. Confining mites in 12 mm diameter arenas surrounded by sticky insect trap adhesive (Davis Gelatine N.Z. LTD., Christchurch) prevented run-off from the leaves. Up to 25 mites were placed into each arena. At least four replicates for each concentration were tested (one replicate per leaflet). Successive experiments were done with larvae, protonymphs, deutonymphs, and adults. Controls with a similar number of water-treated mites were included in tests at each stage.

On experiments where leaf discs were used, five discs (12 mm diameter) were placed on moistened cotton 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 16:8 (L:D) photoperiod until the control 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 leaflets were sprayed in the Potter tower with a range of five thuringiensin concentrations; applications were made from lowest to highest concentration. Later controls were water-treated. To determine the residual toxicity of thuringiensin to *T. urticae*, the mites were placed into the arenas after residues had been 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 the arenas were used: glass Petri dishes that would prevent feeding, and French dwarf bean leaflets that would allow feeding. One thuringiensin concentration was used (0.005 g AI L^{-1}) to test the response of mites on both substrates. Groups of 25 larvae were placed on each arena after the residues had been dried for 15 min. After 8 h, larvae were removed and placed on residue-free leaflets. To determine the effect of direct spray only, 4 groups of 25 larvae were placed on glass Petri dishes, sprayed (0.005 g AI L^{-1}) and transferred immediately to arenas on fresh residue-free French dwarf bean leaflets. Each arena was considered as a replicate (one replicate per leaflet or Petri dish).

Fecundity. The effect of thuringiensin on *T. urticae* fecundity was determined by exposing females to direct sprays or residues for varying periods. Twenty 1-d-old females were placed on French dwarf bean leaflets 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 L^{-1}) 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 leaflet). 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 above experiments. The fecundity of individual females and egg hatch was assessed daily. Each female was considered 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 L^{-1}). Thuringiensin was applied to whole French dwarf bean leaves with a Potter tower, and a water-treated control 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 a replicate; 10 replicates were used for each concentration tested. Leaves were held at 23 ± 2 °C and a 16:8 (L:D) photoperiod until instantaneous mortality rates were assessed as indicated before. 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 $i = (\ln N_t - \ln N_o)/t$, where $\ln N_t$ = natural log of final total population, ln No = natural log of initial population and t = time that elapsed between the initial and final population (7 d). In addition, the percentage of actual 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). This program (POLO) was used to test the goodness-of-fit to the probit model based on a χ^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 lethal concentration (LC) values were measured by using a 95% confidence interval (CI) for the ratio of two values (Robertson and Preisler, 1992). When comparison of the differences between treatments in other experiments was necessary, the natural or transformed data (arcsin $\sqrt{x}$) 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 on *T. urticae*
Effects of direct spraying on the mortality of larvae, protonymphs, deutonymphs and adults are shown in Table 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.

Table 1. Direct and residual toxicity of thuringiensin on different *T. urticae* life stages
Cuadro 1. Toxicidad directa y residual de thuringiensin sobre diferentes estados de desarrollo de *T. urticae*

Treatment	Stage	n	g AI L^{-1}	LC_{50} * 95% CI	g AI L^{-1}	LC_{90} * 95% CI	(SEM)	Slope χ^2	df
Direct	Larvae	901	0.0011	0.0006-0.0016	0.0054	0.0037-0.0102	1.8 (0.1)	9.32	3
	Protonymphs	860	0.0010	0.0004-0.0019	0.0050	0.0025-0.0187	1.9 (0.1)	13.16	3
	Deutonymphs	1752	0.0066	0.0032-0.0185	0.0887	0.0274-1.8700	1.1 (0.7)	20.22	3
	Adults	313	0.5360	0.3514-0.7372	2.8614	1.7875-7.3809	1.8 (0.4)	1.68	3
Residue	Larvae	745	0.0028	0.0020-0.0036	0.0076	0.0054-0.0146	2.9 (0.3)	4.51	3
	Protonymphs	661	0.0138	0.0104-0.0175	0.0521	0.0395-0.0768	2.2 (0.3)	1.04	3
	Deutonymphs	1002	0.0654	0.0092-0.3489	1.8221	0.3432-223.91	0.9 (0.1)	5.91	4
	Adults	580	2.7886	0.3974-9.6972	41.064	11.189 -15372.	1.1 (0.1)	8.93	3

*LCs estimated by probit analysis POLO (Russell *et al.*, 1977).
n: number of individuals; LC: lethal concentration; AI: active ingredient; CI: confidence interval; SEM: standard error means.

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 and Preisler, 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 deutonymphs (16-fold) and adults (530-fold).

Direct toxicity of thuringiensin on *P. ulmi*

Effects of direct sprays on the mortality of larvae, protonymphs, deutonymphs and adults are shown in Table 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 and Preisler, 1992). At the LC_{50}, larvae were significantly more susceptible to 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 (22.3×10^3-fold).

Residual toxicity of thuringiensin to *T. urticae*

Effects of exposure of *T. urticae* to various residues of thuringiensin are shown in Table 1. 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 (5403-fold).

Table 2. Direct toxicity of thuringiensin on different *P. ulmi* life stages
Cuadro 2. Toxicidad directa de thuringiensin sobre diferentes estados de desarrollo de *P. ulmi*

Stage	n	LC_{50} * g AI L^{-1}	95% CI	LC_{90} * g AI L^{-1}	95% CI	Slope (SEM)	χ^2	df
Larvae	1163	0.00002	0.000019-0.00002	0.00004	0.00003-0.00004	5.64 (0.55)	0.47	2
Protonymphs	986	0.00006	0.00005 -0.00007	0.00017	0.00014-0.00021	2.94 (0.20)	3.29	3
Deutonymphs	1201	0.00018	0.00013 -0.0002	0.00068	0.0005 -0.0011	2.24 (0.13)	7.31	3
Adults	930	0.07093	0.03013 -0.12359	0.88954	0.49035-2.37990	1.17 (0.11)	3.88	3

*LCs estimated by probit analysis POLO (Russell *et al.*, 1977).
n: number of individuals; AI: active ingredient; CI: confidence interval; LC: lethal concentration; SEM: standard error means.

Separation of direct, residual and feeding toxicity

Table 3 shows that mortality of *T. urticae* larvae exposed to direct spraying, residues and residues-only was higher on Petri dishes than on leaves. The combined action of direct spraying 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 surfaces where feeding was possible (23%). Mortality from direct spraying without feeding (18.3%) was similar to residue exposure with leaf feeding (23%).

Table 3. The effect of direct, residual, and feeding toxicity of thuringiensin (0.005 g AI L^{-1}) on *T. urticae*

Cuadro 3. Efecto directo, residual y de alimentación de thuringiensin (0.005 g AI L^{-1}) sobre *T. urticae*

Substrate Treatment	n	Mean % mortality (±SEM)
Petri dish (no feeding possible)		
Direct + Residue	120	95.3 (2.1)a*
Residue	125	54.7 (2.4)b
Direct	120	18.3 (5.8)c
Control (water)	130	0.7 (1.2)d
Leaf (feeding possible)		
Direct+Residue	125	52.7 (4.5)b
Residue	120	23.0 (8.7)c
Control (water)	125	0.7 (1.3)d

ANOVA was done using untransformed % mortality values.
*Means with same letters are not significantly different ($P > 0.05$, Tukey's test, Zar, 1984).
n: number of individuals; SEM: standard error means.

Fecundity and egg hatching

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

Population development

Data in Table 5 shows that thuringiensin caused 13.1-, 33.6- and 125-fold higher increases than the control in the instantaneous mortality rate (i) of *T. urticae* at 0.00015, 0.0003 and 0.003 g AI L^{-1}, respectively. Larvae experienced the highest mortality for all concentrations. Total actual mortality ranged between 18.4 - 100% from the lowest to highest thuringiensin concentration.

DISCUSSION

Slow action of thuringiensin has been accepted as a typical feature of this toxicant (Krieg, 1968; Hall *et al.*, 1971; Perring and Farrar, 1986; Royalty and 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

Table 4. Effect of different periods of exposure to residues and direct spraying of thuringiensin on egg laying and hatching of *T. urticae*

Cuadro 4. Efecto de diferentes períodos de exposición a residuos y aplicación directa de thuringiensin sobre la ovipostura y eclosión de *T. urticae*

	Exposure				
	Residue only		Direct + residue		Comparison of mean egg/fem/d between exposure methods
Period (d)	Mean eggs/fem/d (±SEM)	% Hatch (±SEM)	Mean eggs/fem/d (±SEM)	% Hatch (±SEM)	
Untreated	8.64 (0.91)a[1]	90.0 (5.78)a	7.04 (1.17)a	92.7 (4.12)a	=[2]
1	7.06 (2.13)ab	90.1 (5.21)a	5.27 (1.98)ab	84.4 (12.43)a	=
2	5.19 (1.87)bc	87.8 (7.45)a	3.44 (2.10)b	68.0 (24.39)a	=
3	4.01 (1.22)cd	85.2 (8.61)a	1.06 (1.54)c	70.7 (17.00)a	≠
4	0.61 (0.98)e	74.2 (20.8)a	0.34 (1.08)e	74.5 (33.22)a	=

[1]Means with same letters are not significantly different (P > 0.05, Tukey's test, Zar, 1984).
[2]t-test (P = 0.05). Symbol (=) indicates no significant difference between exposure methods.
SEM: standard error means; d: day; fem: female.

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

Cuadro 5. Efecto de thuringiensin sobre poblaciones de *T. urticae*

Thuringiensin concentration g AI L^{-1}	Number of each life stage surviving (% actual mortality of each life stage)					Instantaneous mortality rate (i)	Total % actual mortality
	Eggs	Larva	Proto	Deuto	Adult		
Control	256 (1.2)	253 (0)	253 (0)	253 (0)	252 (0)	-0.0022	1.6
0.00015	207 (12.6)	181 (5.3)	170 (0.5)	169 (0)	169 (0)	-0.0289	18.4
0.0003	270 (3.7)	260 (29.3)	181 (7.8)	160 (0)	160 (0)	-0.0740	40.8
0.003	262 (0.8)	260 (50.8)	127 (32.4)	42 (5.3)	28 (0)	-0.2758	89.3
0.03	201 (0.5)	200 (99.5)	0	0	0		100

AI: active ingredient.

hexythiazox (Welty *et al.*, 1988) and when *P. citri* were treated with cycloprate (Asano and 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. 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 they 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 and Bond, 1973a,b).

To avoid misinterpretation on the toxicological effects of chemicals that affect the metabolic processes involved in moulting, the use of an appropriate mortality assessment criterion and an accurate concentration-response range are necessary. Identification and separation of the mortality that occurs at the moulting stage 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 immature stage of development had previously been shown to not be 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). This trend was less evident with direct exposure because the responses of larvae and protonymphs were similar (Table 2). However, these results clearly demonstrate that early immature stages are more susceptible to thuringiensin than are the later immature and adult stages. With *P. ulmi* LC values significantly increased with the developmental stage, a result that 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 a combination of any or all of these factors.

Robertson and 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 (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 (Table 1). 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 found 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 were achieved.

The effectiveness of thuringiensin varied markedly between *T. urticae* and *P. ulmi* (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 and Bond 1973a,b). In addition, different degrees of susceptibility to thuringiensin may be due to different ratios of thuringiensin to ATP at specific developmental stages between 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 and Preisler, 1992).

Exposure of *T. urticae* to residues or direct spraying 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 on 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 and Worner (1990), population toxicology is far more realistic than prediction based on the response of any stage. Because the field rate could be higher than the concentration levels tested here, and 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 spraying or residues. Both exposure methods may also achieve considerable reductions in fecundity. 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 and Worner, 1990).

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