INVESTIGACIÓN

FACTORS INFLUENCING THE RESPONSES OF *Tetranychus urticae* KOCH (ACARINA: TETRANYCHIDAE) TO THURINGIENSIN[1]

Factores que influyen en la respuesta de *Tetranychus urticae* Koch (Acarina: Tetranychidae) a thuringiensin

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ABSTRACT

The effects of temperature, host plant, active ingredient mobility, surfactant, residue age and larval age on the response of *Tetranychus urticae* Koch to thuringiensin were evaluated in laboratory bioassays. *T. urticae* larvae were significantly more susceptible to thuringiensin at 13 °C than at 28 °C. A significantly different response of *T. urticae* larvae to thuringiensin residues was found on peach and apple leaves. 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; the leaf surface (upper or lower) and the addition of Silwet L-77 surfactant did not affect the efficacy of thuringiensin. The use of thuringiensin in spider mite control programmes is discussed. Practical suggestions on the development of bioassays for active ingredients to assess the effects of environmental factors of this type are also discussed.

Key words: β-exotoxin, miticide, bioassay, pesticide, residue age, surfactant, translocation.

RESUMEN

La actividad de thuringiensin sobre *T. urticae* fue evaluada en bioensayos de laboratorio determinando los efectos de temperatura, planta hospedera, movilidad del ingrediente activo, surfactante, edad del residuo y edad larval. Las larvas de *T. urticae* fueron significativamente más susceptibles a thuringiensin a 13 °C que a 28 °C. Una repuesta significativamente diferente presentaron las larvas de *T. urticae* a los residuos de thuringiensin cuando fue aplicado en hojas de duraznero y manzano. Las larvas más viejas de *T. urticae* fueron significativamente más susceptibles que las larvas jóvenes cuando fueron expuestas a residuos de thuringiensin. No hubo efecto sistémico o translaminar de thuringiensin, la superficie de la hoja y el surfactante Silwet L-77 no afectaron la eficacia de thuringiensin. El uso de thuringiensin en programas de control de arañitas es discutido. La práctica y desarrollo de este tipo de bioensayos también es discutido. Las sugerencias prácticas en el desarrollo de bioensayos para ingredientes activos y la medición de los efectos en los factores medioambientales también son discutidos.

Palabras clave: β-exotoxin, acaricida, bioensayo, pesticida, residuo, surfactante, translocación.

[1]Recepción de originales: 10 de Abril de 2000.
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INTRODUCTION

Thuringiensin, a formulation of β-exotoxin of *Bacillus thuringiensis* Berliner, has been reported to have significant potential for control of mites (Neal *et al.*, 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 maximize 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 (Everson and Tonks, 1981; James *et al.*, 1988). In contrast, responses of mites to dicofol and tetradifon have correlated negatively with temperature (Hassan *et al.*, 1970). No information about the effects of temperature on the response of spider mites to thuringiensin has yet been published.

The response of arthropods to pesticides can also be influenced by the host plant. For example, Wakou and Sugawara (1974) found that the response of *Tetranychus urticae* Koch eggs to dicofol on peach (*Prunus persicae* Sieb and Zucc), bean (*Phaseolus* sp.) and apple (*Malus* sp.) leaves was affected by the relationship between leaf surface and the amount of residues deposited. Asano and Kamei (1982) also showed that response of *Panonychus citri* (McGregor) and *T. urticae* eggs to cycloprate varied with different host plants tested. Similarly, Marris and Chapman (1987) found that hexythiazox caused greatest mortality to *T. urticae* eggs laid on broad bean (*Vicia faba* L.) leaves and lowest mortality to eggs laid on apple leaves. An intermediate mortality was observed for eggs on raspberry (*Rubus idaeus* L.) and strawberry (*Fragaria ananassa* Hort.) 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 maximize the effectiveness of a pesticide, mobility through the plant or over the surface is often desirable. Munthali and Wyatt (1986) demonstrated the importance 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 (Nippon Soda Co., 1984), an ovicidal miticide, also has been shown to having mobility on the leaf surface. Although Mersie and Singh (1988) showed that thuringiensin was absorbed to a limited extent by snapbean (*Phaseolus vulgaris* L. cv. Greencrop) leaves, Neal *et al.* (1987) reported that thuringiensin was not translocated in bush lima beans (*Phaseolus lunatus* L.). 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 and 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 a surfactant (X-77) did not markedly increase ^{14}C-labelled thuringiensin penetration into the leaves of snapbeans (Mersie and Singh, 1988).

Persistence of residues also significantly affects the efficacy of many pesticides. Mersie and 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 and *Panonychus ulmi* (Koch) 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 *Tetratnychus cinnabarinus* (Boisduval) (86.7% mortality) occurred after 12 d on lima beans. 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 (Aveyard *et al.*, 1986; Welty *et al.*, 1988; Marshall and 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 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, New Zealand, since 1985 without exposure to pesticides. Mites from that strain were reared on French dwarf beans (*Phaseolus vulgaris* L. cv. Tendergreen) in the laboratory; under a 16:8 (L:D) photoperiod. Temperature and humidity were not controlled but were approximately 21 ± 3 °C and 60 ± 15% (RH). New plants were added to the colony to replace senesing plants when required.

Miticide. An experimental formulation of thuringiensin (ABG-6320, 5% active ingredient (AI), aqueous suspension, Abbott Laboratories, North Chicago, Illinois, USA), was used in all experiments. The physical and chemical properties of thuringiensin have been reported by Sebesta *et al.* (1981).

Bioassays. Unless otherwise noted, a Potter tower (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) was used for applying thuringiensin suspensions in all experiments. Two milliliter of thuringiensin suspension was sprayed with 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 cm^{-2} (the deposit was estimated by weighing aqueous deposits on microscope coverslips).

Since a concentration-mortality response had 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 leaflets were placed upside down on water moistened cotton in Petri dishes (85 mm diameter). The escaping from the leaflets was prevented by confining mites in 12 mm diameter arenas surrounded by a sticky insect trap adhesive (Davis Gelatine Ltd., Christchurch, N.Z.). On leaf disc experiments, five discs (12 mm diameter) were placed on water moistened cotton 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 the twin leaves were considered suitable for use. The escaping from the leaves was prevented by confining mites in arenas (12 mm diameter) on the undersides of leaflets as described for the whole-leaf method. In all experiments, mites were held at 23 ± 2 °C and a 16:8 (L:D) photoperiod 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 response of *T. urticae* larvae to thuringiensin, five concentrations of thuringiensin 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 leaflets). Leaves were held at 13, 18, 23 and 28 ± 2 °C and a 16:8 (L:D) photoperiod until the control group mites reached adulthood. The responses of the larvae held at different temperatures to residues of thuringiensin were analyzed by probit analysis (Russell *et al.*, 1977). The same program was used for testing goodness-of-fit to the probit model by the χ^2 goodness-of-fit test. The hypothesis 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 evaluated on the basis of 95% confidence intervals (CI) for the ratio of two values (Robertson and 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 L^{-1}) were applied to whole French dwarf bean leaflets. A control treated with water was also included. At least 25 larvae were transferred into arenas; four replicates for each treatment were included. Leaflets were held at 13, 18 and 23 ± 2 °C and a of 16:8 (L:D) photoperiod until the control group mites reached adulthood. To compare the differences between treatments, transformed data (arcsin √% mortality) were subjected to a two-way ANOVA. The means were compared with Tukey's test (Zar, 1984).

Residue age. To determine the responses of larvae to different age thuringiensin residues, five concentrations of thuringiensin were applied to whole bean leaves. A control treated with water was also used. Treated leaflets were held in the controlled temperature cabinet at 23 ± 2°C and a 16:8 (L:D) photoperiod until the control group mites reached adulthood. 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 16:8 (L:D) photoperiod until the control group mites reached adulthood. The responses of larvae to different age thuringiensin residues were analysed by probit analysis as described above.

Leaf type. The influence of host plant on the response of *T. urticae* to thuringiensin was studied in three experiments. The effects of leaf type and leaf surface (lower and upper) were tested by applying one concentration (0.00025 g AI L^{-1}) of thuringiensin to French dwarf bean, peach (*Prunus persica* cv. Red Haven, S. and Z) and apple (cv. Red Delicious) leaf discs. A water control 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 16:8 (L:D) photoperiod until the control group mites reached adulthood. To compare the differences between treatments, transformed data (arcsin √% mortality) were subjected to a two-way ANOVA. The means were compared with Tukey's test (Zar, 1984).

Translocation. The translaminar effect of thuringiensin was tested with the twin-leaf method by applying two thuringiensin concentrations (0.0828 and 8.28 g AI L^{-1}) on bean leaves. 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 were applied per leaf. Six-twelve-h-old larvae were placed into arenas (12 mm diameter) on the lower surface of the leaflets and were held at 23 ± 2 °C and a 16:8 (L:D) photoperiod until the control group mites reached adulthood.

The systemic effect of thuringiensin was investigated using the twin-leaf method. One bean leaflet was dipped in a suspension of thuringiensin (8.28 g AI L^{-1}) and Citowett (0.025%). Approximately 0.21 mL was applied per leaflet. Six-twelve-h-old larvae were placed into arenas on the lower side of the untreated leaflet opposite the treated leaflet. For both experiments, at least 20 larvae were used per replicate, with six replicates per treatment. Conditions after treatment and evaluations were similar to those already described. Transformed data (arcsin √% mortality) 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 L^{-1}) 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 per treatment. Leaves were held at 23 ± 2 °C and a 16:8 (L:D) photoperiod until the control group mites reached adulthood. Transformed data (arcsin √% mortality) were subjected to one-way ANOVA and the means were compared with Tukey's test (Zar, 1984).

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 and 18 h) were placed on the residues for 6 h and then transferred to leaflets 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 analyzed by probit analysis as described above.

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 L^{-1}) 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 16:8 (L:D) photoperiod 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 √% mortality) 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. 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 (95% CI). 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)

Table 1. The effect of temperature on the toxicity of thuringiensin to *T. urticae* larvae
Cuadro 1. Los efectos de la temperatura en la toxicidad de thuringiensin sobre larvas de *T. urticae*

Temperature °C	*n*	LC_{50}* (g AI L^{-1})	95% CI	LC_{90}* (g AI L^{-1})	95% CI	Slope (SEM)	χ^2	df
13	1,053	0.00031	0.00021-0.00044	0.00103	0.00069-0.00214	2.45 (0.14)	12.54	3
18	1,114	0.00037	0.00023-0.00056	0.00116	0.00073-0.00281	2.57 (0.14)	17.76	3
23	901	0.00190	0.00064-0.00164	0.00542	0.00327-0.01438	1.84 (0.13)	9.31	3
28	999	0.00137	0.00084-0.00205	0.00504	0.00314-0.01268	2.26 (0.13)	14.59	3

*LCs estimated by probit analysis (POLO; Russell *et al.*, 1977). *n*: number of individuals; LC: lethal concentration; CI: confidence interval; AI: active ingredient; SEM: standard error means; χ^2: chi-square; df: degrees of freedom.

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 very significant (P = 0.01).

Table 2. The response of *T. urticae* larvae to three thuringiensin concentrations at three different temperatures
Cuadro 2. Rspuesta de larvas de *T. urticae* a tres concentraciones de thuringiensin y tres temperaturas diferentes.

Temperature °C	Concentration (g AI L^{-1})	Mean % mortality (±SEM)
13	0.00015	21.5 (± 4.72) b*
	0.003	44.5 (± 22.5) b
	0.03	97.5 (± 0.57) c
18	0.00015	5.25 (± 2.99) a
	0.003	46.8 (± 18.28) b
	0.03	93.3 (± 7.410) c
23	0.00015	4.75 (± 1.50) a
	0.003	36.25 (± 21.8) b
	0.03	55.75 (± 9.95) b

% Mortality data transformed to arcsin √%.
*Means with same letters are not significantly different (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); AI: active ingredient; SEM: standard error means.

Residue age. The responses of *T. urticae* larvae to thuringiensin residues of different ages are shown in Table 3. 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 (95% CI). 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 larval response to residues was relatively consistent up to 8 d, after which time period 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 leaves was significantly (P < 0.05) lower than on peach and apple. Mortality between leaf surface for any leaf type were not significantly different.

Table 3. The toxicity of thuringiensin to *T. urticae* larvae exposed to residues of different ages
Cuadro 3. Toxicidad de thuringiensin sobre larvas de *T. urticae* expuestas a residuos de diferentes edades

Residue age (d)	*n*	LC_{50} (g AI L^{-1})	95% CI	LC_{90} (g AI L^{-1})	95% CI	Slope (SEM)	χ^2	df
1	611	0.00250	0.0023-0.0027	0.00568	0.00495-0.00673	3.55 (0.25)	0.74	3
2	881	0.00335	0.0021-0.0048	0.01445	0.00930-0.03225	2.02 (0.16)	6.81	3
4	803	0.00330	0.0018-0.0046	0.00904	0.00619-0.02412	2.92 (0.86)	16.53	4
8	600	0.00370	0.0033-0.00042	0.00802	0.00697-0.00971	3.91 (0.41)	2.08	3
13	871	0.01080	0.0071-0.01264	0.02319	0.01822-0.03727	3.58 (0.30)	7.89	3
21	564	0.00982	0.0053-0.01378	0.01913	0.01366-0.06352	4.43 (0.39)	6.41	2

LCs estimated by probit analysis (POLO; Russell *et al.*, 1977). (d): day; *n:* number of individuals; LC: lethal concentration; CI: confidence interval; SEM: standard error means; χ^2: chi-square; df: degrees of freedom.

Table 4. The response of *T. urticae* larvae to thuringiensin residues on different leaf types and surfaces
Cuadro 4. Respuesta de larvas de *T. urticae* a residuos de thuringiensin en diferentes tipos de hojas y superficies

Leaves	Leaf surface	*n*	Mean % mortality (±SEM)
Bean	lower	200	75.75 (3.3) a*
	upper	198	72.00 (5.4) a
Peach	lower	195	95.50 (1.0) b
	upper	201	97.00 (1.2) b
Apple	lower	201	94.50 (3.4) b
	upper	198	95.00 (2.6) b

*ANOVA done using arcsin √% mortality. Means with same letters are not significantly different (P > 0.05; Tukey's test [Zar, 1984]). AI: active ingredient; n: number of individuals; SEM: standard error means.

Translocation. 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 (Table 5).

Surfactant. Silwet L-77 did not significantly (P > 0.05) affect mortality when larvae were exposed to thuringiensin residues or when they were sprayed directly (Table 6). 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. 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 (95% CI) less susceptible than were 12-h-old larvae (1.9-fold) and 18-h-old larvae (2.3-fold). At the LC_{90}, 2-h-old *T. urticae* 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.

Table 5. Evaluation of translaminar and systemic activity of thuringiensin on *T. urticae* larvae in French dwarf bean plants
Cuadro 5. Evaluación del efecto translaminar y sistémico de thuringiensin sobre larvas de *T. urticae* en plantas de poroto

Concentration g AI L^{-1}	Test for translaminar action		Test for systemic action	
	n	Mean % mortality (±SEM)	*n*	Mean % mortality (±SEM)
0	111	3.5 (3.8) a*	132	2.3 (2.4) a**
0.0828	109	2.6 (3.2) a	-	-
8.2800	114	2.8 (1.9) a	138	2.9 (2.5) a

*ANOVA done using arcsin √% Mortality. Means with same letters are not significantly different (P > 0.05; [Zar 1984]).
**t-Test; Means with same letters are not significantly different (P > 0.05 [Zar, 1984]).
AI: active ingredient; *n*: number of individuals; SEM: standard error means.

Table 6. The effect of Silwet L-77 surfactant at 0.1% v/v on the response of *T. urticae* larvae to thuringiensin residues (0.00025 g AI L^{-1})
Cuadro 6. El efecto del surfactante Silwet L-77 al 0,1% v/v en la respuesta de larvas de *T. urticae* a los residuos de thuringiensin (0,00025 g IA L^{-1})

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

*ANOVA done using arcsin √% mortality. Means with same letters are not significantly different (P > 0.05; Tukey's test [Zar, 1984]).
AI: active ingredient; n: number of individuals; SEM: standard error means.

DISCUSSION

The negative temperature-toxicity relationship of thuringiensin with larvae (Table 1) and the significant interaction between temperature and concentration (Table 2) may reflect the underlying biochemical mechanism of this compound. 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 between 25-65 °C because the rate of metabolism, and particularly the rate of oxygen consumption, are affected (Rajagopal and Bursell, 1966). 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

Table 7. The response of *T. urticae* larvae of different ages to thuringiensin residues
Cuadro 7. Respuesta de larvas de *T. urticae* de diferentes edades a residuos de thuringiensin

Larval age (h)	*n*	LC_{50}* (g AI L^{-1})	95% CI	LC_{90}* (g AI L^{-1})	95% CI	Slope (SEM)	χ^2	df
2	673	0.00211	0.00148-0.00278	0.00759	0.00533-0.01433	2.3 (0.25)	3.18	3
12	900	0.00109	0.00064-0.00164	0.00542	0.00327-0.01438	1.8 (0.13)	9.31	3
18	678	0.00090	0.00076-0.00107	0.00434	0.00338-0.00594	1.9 (0.14)	3.51	4

*LCs estimated by probit analysis (POLO; Russell *et al.*, 1977).
(h): hora; *n*: number of individuals; LC: lethal concentration; AI: active ingredient; CI: confidence interval; SEM: standard error means; χ^2: chi-square; df: degrees of freedom.

Table 8. The effects of three thuringiensin concentrations on *T. urticae* larvae of two ages
Cuadro 8. Efectos de tres concentraciones de thuringiensin en larvas de dos edades de *T. urticae*

Larval age (h)	Concentration g AI L^{-1}	Mean % mortality (±SEM)
2	0.0002	8.20 (± 6.14) a*
	0.001	11.18 (± 6.21) ab
	0.005	31.10 (± 17.77) b
18	0.0002	11.85 (± 13.61) a
	0.001	22.33 (± 14.84) ab
	0.005	75.58 (± 19.24) c

% Mortality data transformed to arcsin √%.
*Means with same letters are not significantly different ($P > 0.05$; 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 larval age ($F = 3.6$, df = 2.18, $P = 0.05$).
(h): hora; AI: active ingredient; SEM: standard error means.

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; Neal *et al.*, 1987; Mersie and 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 (Vargas *et al.*, 2001), suggests that significant control of field populations of mites should be achieved. However, further assessment of the efficacy of thuringiensin against field populations while taking into account the phenology of each species (Robertson and 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 leaves (Table 4). Many factors including leaf surface structure and physiology and the amount of chemical deposited could affect thuringiensin uptake and mortality (Wakou and Sugawara, 1974; Asano and Kamei, 1982; Marris and 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 so 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* in order 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 and Mersi, 1989). However, results in Table 5 suggest that sufficient thuringiensin does not penetrate into the leaves 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 did not aid the penetration of thuringiensin into the leaf cuticle or epidermal cells and thereby 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 the organosilicone 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 and 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 and Couble, 1979). The increasing susceptibility of *T. urticae* larvae with age (Table 7) 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 used in bioassays 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.

ACKNOWLEDGMENTS

We would like to thank J.L. Robertson, Forest Service Pacific Southwest, Forest and Range Exp. St., Berkeley, USA, and Ms. S. P. Worner, Lincoln University, Canterbury, New Zealand, for their valuable comments and discussions on earlier versions of the manuscript and help with statistical interpretation, and A. Ubillo, Instituto de Investigaciones Agropecuarias, Centro Experimental de Entomología La Cruz, Chile, for reviewing and format adaptation to Agricultura Técnica (Chile).

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