Experimental and Applied Acarology **26:** 71–78, 2002.

Effects of triflumuron on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranichydae)

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Received 13 June 2001; accepted in revised form 28 January 2002

Key words: Acari, Chitin, Insect Growth Regulator, Spider mite, *Tetranychus urticae*, Triflumuron

Abstract. The effects of triflumuron on the mortality, fecundity, and fertility of the two-spotted spider mite, *Tetranychus urticae,* were evaluated in the laboratory. No differences in toxicity for larvae, protonymphs and deutonymphs were observed, but immature stages were 3.8-times more susceptible than adults at the LC_{50}. The compound exhibited a direct contact ovicidal activity influenced by eggs age. 48–72 h old eggs were significantly more sensitive than eggs of the other age classes. No hatch inhibition was observed in eggs laid by treated adult females using a sublethal dose at two physiological times (<12 and 48–72 h old). However, fecundity decreased in younger treated females, but it increased in the older-ones. The toxicity for immatures and eggs, and the sublethal effects described suggest that triflumuron could be an interesting incorporation in integrated pest programs of *T. urticae*.

Introduction

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) has become increasingly evident in the last few decades, after broad spectrum pesticides have been applied. It has been recorded on more than 150 hosts of some economic value throughout the world (Jeppson et al. 1975). There is no doubt that this species is the most polyphagous spider mite of the tetranychids (Van de Vrie 1985), being a key pest for many kinds of crops in temperate regions.

Currently, great efforts are directed towards a reduction in the use of traditional pesticides and towards an increase in the use of Integrated Pest Management (IPM) techniques. Therefore, the search for pesticides that are compatible with IPM programs, such as benzoylphenyl ureas (BPU's) is an interesting approach (Granett 1987).

BPU's inhibit chitin synthesis in a wide range of insect groups, resulting in abortive moulting. They act mainly as larvicides and ovicides (Retnakaran and Wright 1987). Effects on adult fecundity, fertility, and longevity have also been reported (Pickens and De Milo 1977; Ivie and Wright 1978; Koehler and Patterson 1989; Haynes and Smith 1993; Marco and Viñuela 1994; Marco et al. 1998). Acaricidal activity was also mentioned by several authors for various mites, including *T. urticae* (Grosscurt et al. 1988; Scheltes et al. 1988; Ahn et al. 1993). However,

although BPU's have a low toxicity for mammalians and are relatively harmless for spider mite natural enemies (Scheltes et al. 1988; Mansour et al. 1993; Park et al. 1996), little information is available about their effects on this kind of pest.

Our studies were carried out to determine the effects of the BPU triflumuron on the mortality of two-spotted spider mite in different life stages: eggs, larvae, protonymphs, deutonymphs and adults. We also evaluated the effects on adult fecundity and fertility. Finally we consider the possibility of incorporating triflumuron in the management of *T. urticae*.

Materials and Methods

Colony source

A laboratory colony was used for the bioassays. The colony was collected from a natural population on ornamental crops in 2000 and maintained since then on young pesticide-free green bean plants (*Phaseolus vulgaris,* var. Garrafal). The plants were introduced in acrylic cages (40 by 40 by 55 cm) placed in a climatic chamber at 27±1°C, 70±10 % RH, and 16:8 (L: D). Bioassays were performed under the same conditions.

Synchronous cohorts

When it was necessary to synchronize the developmental stages, about 50 females from the nucleus colony were placed, with a camel brush, in single green bean leaves on wet filter paper inside a Petri dish, allowing them to lay eggs for 12 h.

Chemical

The BPU triflumuron was used in the bioassays as the available form Alsystin® ([wettable powder] 250 g [ai] / kg), commercialized by Bayer Hispania (Spain).

Effects on larvae, protonymphs, deutonymphs and adults

Larvae, protonymphs, deutonymphs and adults newly emerged were treated in respective bioassays. In each case, mites were placed on green bean leaf disks of 2 cm diameter over wet filter paper in a Petri dish to maintain the leaf humidity and to avoid the escape of the mites. In order to prevent an excess of humidity, two holes of 6 mm diameter were made on the top of the Petri dishes.

Every bioassay consisted of 5 replicates per dose level and the distilled water treated control, each containing 10 individuals. Dosages for each stage were from 0.25 to 16 g/l on larvae, 0.04 to 6.00 g/l on protonymphs, 0.05 to 26.26 g/l on deutonymphs, and 0.75 to 12.00 g/l on adults. These dosages were chosen after preliminary bioassays.

Insecticide applications were made on leaf disks with the spider mites, using a hand sprayer. The amount of mixture deposited was 9.6 ± 0.83 ml/cm^2.

The mortality data used in the analysis were those recorded 6 days after treatment.

Ovicidal effects

Four *T. urticae* females were placed in each leaf disk used in the bioassay and allowed to lay eggs for 12 hours. The eggs layed (about 15 per leaf disk) were treated as above, at the following age groups: 0-24, 24-48, 48-72, and 72-96 h old. The doses employed for each age class were 2 and 8 g/l of compound, with 5 replicates per dose and the control was treated only with distilled water. Egg hatch was recorded after 6 days of treatment.

Effect on adult fecundity and fertility

Adult females were treated as above with a dose of 0.75 g/l of compound at two physiological points: < 12 h old (Y) and 48–72 h old (O). After treatment each female was transferred to a separated untreated disk, with two males (in order to mate, if necessary). After each day of oviposition, females were passed to other untreated leaf disks until death. The eggs on each leaf disk were counted and incubated to assess the hatching percentages. Twelve replicates of each female age class and the control (treated with distilled water only) were made.

Statistical methods

LC values of triflumuron against the different spider mite stages, except egg and 95% fiducial limits, were obtained by probit analysis using the computer program POLO-PC (LeOra Software 1987). A parallelism test was performed according to the relative potency estimation method. The criterion used to estimate the differences between LC ratios was based on their 95% Confidence Interval (Robertson and Preisler 1992).

The significance of the results (egg mortality, fecundity and fertility) was tested by one-way analysis of variance (ANOVA) and means were separated by a LSD multiple range test ($P < 0.05$) using the computer program Statgraphics (Robertson and Preisler 1992).

Results

Effects on larvae, protonymphs, deutonymphs and adults

Toxicity data from larvae, protonymphs, deutonymphs and adults allowed us to estimate the mortality probit regression lines. For larvae, protonymphs, and

Table 1. Parameters of the probit-log dose regression lines fitted for *T. urticae* larvae, protonymphs, deutonymphs and adults, sprayed with Alsystin® ([wettable powder] 250 g of triflumuron / kg).

Developmental stage	slope±S.E	intercept±S.E	LC_{50} (g/l) (Fiducial limits 95%)	LC_{90} (g/l) (Fiducial limits 95%)
Immature stages* (except eggs)	0.57±0.07	5.09±0.01	1.18 (0.76, 1.84)	208.23 (72.54, 1082.01)
Adults	0.57±0.07	4.61±0.11	4.44 (2.38, 8.53)	791.30 (290.18, 3135.90)

*Larvae, protonymph and deutonymph have the same line (χ^2=0.70, g=0.05).

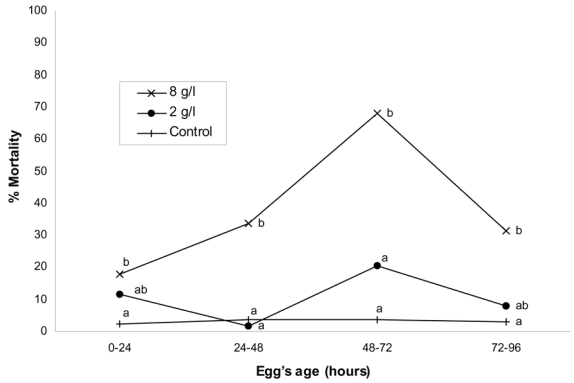

Figure 1. Influence of the egg's age on the direct contact ovicidal activity of Alsystin® ([wettable powder] 250 g of triflumuron / kg) in *T. urticae* for different doses. Within each age class, the data followed by the same letter do not differ significantly at the 5% level (ANOVA and LSD).

deutonymphs the hypothesis that the slopes and the intercepts are the same was accepted having the same regression line. The probit-log dose line fitted for adult mortality was different but had the same slope as the one fitted for immature stages (Table 1).

Ovicidal effects

The effectiveness of triflumuron on eggs of different age classes is shown in Figure 1. The efficacy of the compound depended on the age of the eggs. Eggs less than 24 h-old, 24–48 h, and 72–96 h old were slightly affected at the tested doses. Surprisingly, a high suppression of egg hatching was obtained when treating 48–72 h old eggs. For 72–96 h old eggs, however, the effectiveness was again reduced, and a similar value to that obtained for young eggs was found. Only at the highest tested dose, triflumuron affected egg mortality inside each age classes. Statistical significant differences were not scored between the lower dose and the control.

Table 2. Cumulative number of eggs per female and percentages of eclosion in *T. urticae* when adults were sprayed only with water (control) and with 0.75 g/l of Alsystin® ([wettable powder] 250 g of triflumuron / kg) in two physiological times. (Data are means of twelve replicates ± SE).

Treatment	Eggs/♀	% Eclosion
Control	39.3^{ab}±6.9	98.9^{a}±0.5
< 12 h old	27.8^{a}±7.8	96.5^{a}±1.4
48–72 h old	54.2^{b}±7.2	94.7^{a}±1.2

Within the columns, the data followed by the same letter do not differ significantly at the 5% level (ANOVA and LSD).

Effect on adult fecundity and fertility

Table 2 shows the cumulative number of eggs per female during their life span, and the percentages of eggs hatching. A high intrinsic variability in the number of eggs laid per female was recorded in our bioassays and statistically significant differences were not scored.

Concerning fertility, no effect was observed. High percentages of egg hatching (>94%) were scored in both treated and untreated females.

Discussion

There are BPU's, such as flucycloxuron and flufenoxuron, that showed to be promising acaricides against different spider mite pests like *Tetranychus* spp. and *Panonychus* spp. (Scheltes et al. 1988; Grosscurt and Wixley 1991; Grosscurt et al. 1988; Ahn et al. 1993). However, the acaricidal activity of triflumuron has been scarcely reported for phytophagous mites; i.e., Raizer et al. (1988) observed a slight reduction in the population levels of the mite *Brevipalpus phoenicis* after field triflumuron treatments against the control. However, in our laboratory study, triflumuron exhibited acaricidal activity against *T. urticae*.

The effect of triflumuron on *T. urticae* mortality was related to the developmental stage. Larvae, protonymphs and deutonymphs were equally susceptible, and 3.8-times more susceptible than adults. Ahn et al. (1993) observed that flufenoxuron also exhibited a stronger activity against larvae than adults. This is a result to be expected due to the mode of action of BPU's above indicated.

Triflumuron also exhibited ovicidal activity on *T. urticae*. The efficacy of the compound depended on the age of the eggs. Grosscurt et al. (1988) observed that the ovicidal contact activity of flucycloxuron on the two spotted spider mite was negatively correlated with the age of the eggs treated. In contrast with this result, we observed a significantly higher sensitivity in 48–72 h old eggs towards triflumuron. At 28°C, approximately 68 h after oviposition, the perforation organs (one of the main components of the respiratory system of the *T. urticae* embryos) penetrate the egg shell (Crooker 1985). Dittrich and Streibert (1969) suggested that

this establishment of the respiratory system makes the egg more susceptible to poisoning by certain acaricides, which is consistent with our results.

Egg mortality also depended of the dosage employed. At 2 g/l no effect was observed, however at 8 g/l, triflumuron had ovicidal activity reaching a 68% mortality on eggs 48–72 h old.

When a sublethal dose of triflumuron was applied to adult females in two different physiological times (new emerged and 48–72 h old) no effect on fertility was observed. Egg hatch inhibition after adult treatment with triflumuron has been reported for different insect species, especially Diptera as *Musca domestica* (Howard and Wall 1995), *Bactrocera oleae* (Mazomenos et al. 1997), and *Ceratitis capitata* (Casana-Giner et al. 1999). Nevertheless, no studies about the effect on fertility for spider mites have been reported. Furthermore, this activity has been rarely studied for other BPU's; Grosscurt et al. (1988) observed an ovicidal activity of PH 70–23 (flucycloxuron) against *T. urticae* as a consequence of a transovarial transmission of the compound. Ahn et al. (1993) also observed a substantial reduction in egg viability when treating *T. urticae* deutonymph females with flufenoxuron. Other kinds of Insect Growth Regulators such as azadirachtin also caused a reduction in the percentage of eggs hatched when the compound was applied to adult *T. urticae* females (Dimetry et al. 1993).

Triflumuron did not affect female fecundity. Nevertheless it seems that there was a reduction in the fecundity of young treated females compared to the ones treated 48 – 72 hours after ecdysis (Table 2). Various authors have observed that triflumuron stimulates the fecundity of mites, such as the North American house-dust mite *Dermatophagoides farinae*, when females taken at random from a laboratory culture are treated (Downing et al. 1990), and *T. cinnabarinus* when treated females of 48–72 h old (Mansour et al. 1993). Therefore, the physiological reproductive stage of the females treated seems to be a key factor in order to determine the effect on this important biological parameter.

More laboratory, semi-field and field testing is required before the acaricidal potential of triflumuron can be considered completely investigated, but our studies suggest that the combination of the toxic and sublethal effect produced could lead to the incorporation of this compound in Integrated Pest Management programs against *T. urticae*.

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