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Influence of sublethal exposure to triflumuron on the biological performance of *Tetranychus urticae* Koch (Acari: Tetranychidae)

F. J. Sáenz-de-Cabezón[1], E. Martínez-Villar[2], F. Moreno[2], V. Marco[2] and I. Pérez-Moreno[2]*

[1] *Department of Entomology. University of California Davis. One Shields Avenue. Davis. CA. 95616. USA*
[2] *Departamento de Agricultura y Alimentación. Universidad de La Rioja. C/ Madre de Dios 51. 26006-Logroño. Spain*

Abstract

Effects of sublethal exposure to triflumuron on the biological performance of the two-spotted spider mite *Tetranychus urticae* Koch were analysed under laboratory conditions. Survivorship was affected by the compound. Triflumuron caused a reduction both in the percentage of eggs that developed to adults and in the survival of adult stage. Triflumuron also affected the fecundity. The net reproductive rate (R_0), the intrinsic rate of increase (r_m), and the finite rate of increase (λ) of treated females were lower than in those non treated, resulting in a reduction of population growth. These results suggest that triflumuron could be a valuable addition in integrated pest management programs of *T. urticae,* although more laboratory, semi-field and field testing is required.

Additional key words: benzoylphenyl ureas, intrinsic rate of increase r_m, life-table, triflumuron, two-spotted spider mite.

Resumen

Efecto de una exposición subletal de *Tetranychus urticae* Koch (Acari: Tetranychidae) al triflumurón sobre sus parámetros biológicos

Se analizaron, bajo condiciones de laboratorio, los efectos de una exposición subletal al triflumurón sobre los parámetros biológicos de la araña amarilla *Tetranychus urticae* Koch. La supervivencia fue afectada por el compuesto. El triflumurón causó una reducción tanto en el porcentaje de huevos que llegaron a adultos como en la supervivencia de los imagos y en la fecundidad. La tasa reproductiva neta (R_0), el coeficiente de incremento intrínseco (r_m), y el coeficiente de incremento finito (λ) de las hembras tratadas fueron más bajos que en las no tratadas, dando como resultado la reducción del crecimiento de la población. Estos resultados sugieren que el triflumurón podría ser una incorporación interesante en programas de manejo integrado de *T. urticae,* aunque es preciso desarrollar más experimentos tanto de laboratorio como de semicampo y campo.

Palabras clave adicionales: araña amarilla, benzoilfenil ureas, coeficiente de incremento intrínseco r_m, tabla de vida.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) has been recorded on more than 150 hosts of some economic value throughout the world (Jeppson *et al.,* 1975), being the most polyphagous spider mite of the tetranychids and a key pest for many kind 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 benzoylphenil ureas (BPUs) is an interesting approach.

BPUs 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 adults fecundity, fertility, and longevity have also been reported (Marco *et al.,* 1998; Perveen, 2000; Medina *et al.,* 2002). Acaricidal activity

* Corresponding author: ignacio.perez@daa.unirioja.es
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has been also mentioned by several authors for various spider mites, including *T. urticae* (Grosscurt *et al.,* 1988; Scheltes *et al.,* 1988; Ahn *et al.,* 1993; Sáenz-de-Cabezón *et al.,* 2002, 2003), but little information is available about their effects on this kind of pests.

In ecotoxicology, the ability to predict the effects of toxicants on the dynamics of natural populations can be seen as a major objective (Moe *et al.,* 2001). Standardized test procedures have been formulated to estimate critical-effect levels (LC_x, EC_x) from concentration-response relationships for single life cycle variables such as mortality, growth, or reproduction (Kammenga *et al.,* 1997). Then, demographic studies allow the integration of several critical life cycle traits into a single variable (Van Leeuwen *et al.,* 1985). Several authors have argued that the best approach for evaluation of the total effect of a xenobiotic is life table analysis or demographic toxicology (Stark and Wennergren, 1995). Then, the use of the intrinsic rate of increase (r_m) has been recommended (Allan and Daniels, 1982), because it is based on both survivorship and fecundity. This parameter is also a measure of the ability of a population to increase in an unlimited environment (Stark and Wennergren, 1995).

Triflumuron (like other BPUs) can be slow acting and exert sublethal as well as lethal effects. Then, the compound can affect more than one life stage and pesticide persistence is an important consideration when trying to estimate their effects. Therefore, in this work, the effects of a sublethal dose of triflumuron against *T. urticae* were evaluated using demographic toxicological analysis. The possibility of incorporating this compound in the management of the spider mite was discussed.

Material and Methods

Colony source

A laboratory colony was used for the bioassay. 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,* cv. Garrafal). The plants were introduced in acrylic cages (40 by 40 by 55 cm), placed in a climatic chamber at 24 ± 1°C, 65 ± 5% RH, and 16:8 (L:D). The bioassay was performed under the same conditions.

Chemical

The commercial formulation of BPU triflumuron, namely Alsystin® ([wettable powder] 250 g [ai] kg^{-1}), Bayer Hispania (Spain), was used for the bioassay.

Application of insecticide

One newly ecdysed adult female was placed on each one of the green bean leaf discs (2 cm diameter) used in the bioassay and provided with two adult males. Eight leaf discs with the mites were introduced in each rearing unit consisting of wet filter paper inside a Petri dish (9 cm diameter). Dish top had two holes of 6 mm diameter each to prevent moisture. The rearing units were treated using a Potter Precission Laboratory Spray Tower (Potter, 1952), with 5.5 ml of an aqueous solution of 1g L^{-1} of Alsystin®. An air compressor (Burkard 0523-703 Q-R32X; 50 kPa) was used to apply the insecticide. This resulted in a homogeneous spray coverage of 5 ± 0.5 µl (mean ± SE) fluid per square centimeter. Controls were treated with distilled water alone. The concentration was chosen based on previous bioassays (Sáenz-de-Cabezón *et al.,* 2002), because it has a lower short term effect than the LC_{50} against the more susceptible developmental stage of the mite.

Life-table parameters

Adults were maintained in the same leaf discs throughout the whole bioassay. Daily records for preoviposition and oviposition periods and fecundity were made. Life table parameters were taken until the death of the last individual. In order to obtain a non detectable error doubt to the bioassay, 13 rearing units were sprayed with the compound and 13 with distilled water according with Rejman and Jesiort (1977).

To examine any possible carry-over and residual activity of triflumuron on the offspring of treated adults, 100 eggs from randomly selected females of each treatment were transferred individually to green bean leaf discs treated with the same methodology as described above. Percentage hatchability, mortality,

Table 1. Definition and formulas for nine life table parameters of *Tetranychus urticae*

Symbol	Definition	Formula
x	Age	
l_x	Probability of an individual surviving to age x	
m_x	Reproductive expectation of a female at age x	
GRR	Gross reproductive rate: expected total number of female births produced by a female who lives through all ages	Σm_x
R_0	Net reproductive rate: number of daughters that replace an average female in course of a generation	$\Sigma l_x m_x$
T	Mean generation time: mean of the period over wich progeny are produced	$\Sigma x\ l_x m_x$
r_m	Intrinsic rate of increase: number of progeny produced per unit of time	$(log_e R_0)/T$
ψ	Finite rate of increase: number of times a population multiplies itself in unit time	e^{rm}
DT	Doubling time	$(log_e 2)/r_m$

time of development of each stage, and days to adulthood were monitored once a day.

Statistical methods

Once determined the preoviposition period, the prereproductive survival and the emergence matrixes, r_m 2.0 program (Taberner *et al.,* 1993) was used to establish the natural parameters. The data analysis was carried out using Bootstrap technique doing 1,000 replicates as suggested by Meyer *et al.* (1986). The chosen sex ratio was 1 male to 3 females, according with Helle and Pijnaker (1985). Nine life table parameters were compiled (Table 1).

Results

Examination of the l_x and m_x (as defined in Table 1) in untreated mites revealed low mortality in the immature stages (20% of eggs and 10% of larval and nymphal stages) with a 70% chance of reaching adulthood. In contrast, treated females showed higher mortality in the immature stages (40% of eggs and 16% of larval and nymphal stages) with a 44% chance of reaching adulthood (Fig. 1). The untreated mites spent half of their lives in the adult stage and did not reach their peak of reproduction (mx = 10.5) until the adults were 9 days old, but the probability of reaching this age was only 5%. The treated mites spent less than half of their lives in the adult stage, and reached their peak of reproduction 4 days after reaching adulthood (mx = 6.0). The probability of reaching this age was only 8.8%. No delay in time reaching reproductive maturity was observed in treated mites. The effects of mortality on the population growth can be demonstrated by simply comparing the net reproductive rate (R_o) (Table 2).

Due to mortality effects, the average treated female only produced 5.0 females, almost three-fold less than untreated mites (14.4). For untreated mites, the mean generation time *(T)* was 13.97 days, and the population increased daily by 1.22 times the previous day's total number (finite rate of increase, λ). Every 3.51 days (doubling time, DT) the population doubled. Generation time of the treated mites did not differ much from generation time of the untreated ones. The population increased daily by 1.13 times. Every 5.73 days the population doubled. Untreated mites had significantly higher r_m values (0.198 ± 0.005) than the treated ones (0.121 ± 0.021), with no overlap of their confidence intervals at 95% (Table 2).

Discussion

The r_m value integrates age at first reproduction, survivorship, brood size and frequency, and longevity. However, it is also worth examining how chronic exposure affects the individual components of r_m. In our experimental conditions, the probability that individuals can reach the peak of reproduction and the physiological maximum of reproduction (l_x and m_x values in that maximum, respectively) was lowered by triflumuron. This caused a reduction in the number of

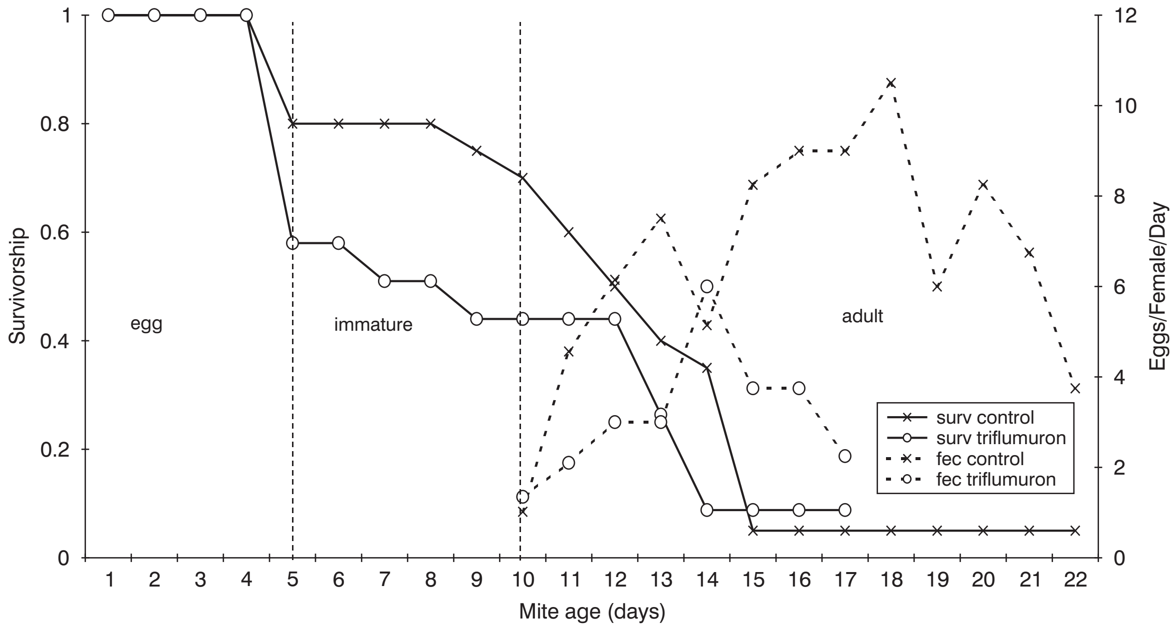

Figure 1. Survivorship (l_x) and fecundity (m_x) curves for *Tetranychus urticae* reared on green bean leaf discs treated with distilled water (control) and with a low mortality concentration (1 g L^{-1}) of Alsystin® ([wettable powder] 250 g of triflumuron kg^{-1}), at 24 ± 1°C, 65 ± 5% RH, and 16:8 (L:D).

daughters that replace an average female in course of a generation (R_o). At this point, is interesting to arrange that the physiological reproductive stage of the females treated seems to be a key factor in order to determine the effect on fecundity (Sáenz de Cabezón *et al.,* 2002). It is appropiate to underline the importance of the mortality in eggs laid by treated females. Egg hatch inhibition after adult treatment with triflumuron has been reported for different insect species, especially Diptera as *Ceratitis capitata* (Wiedemann) (Casana-Giner *et al.,* 1999). Nevertheless, this activity has been rarely studied for BPUs and spider mite pests; 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

Table 2. Life table parameters of *Tetranychus urticae* reared on green bean leaf discs sprayed with 1g L^{-1} of Alsystin® ([wettable powder] 250 g of triflumuron kg^{-1}) at 24 ± 1°C, 65 ± 5% RH, and 16:8 (L:D)

Parameter	Control	Triflumuron
Gross reproductive rate *(GRR)*	85.88	25.20
Net reproductive rate (R_0)	14.40	5.016
Mean generation time (*T*, days)	13.97	13.61
Intrinsic rate of increase (r_m)	0.198 ± 0.005	0.121 ± 0.021
Confidence interval 95%	0.188 – 0.208	0.061 – 0.179
Finite rate of increase (ψ)	1.219	1.129
Doubling time (*DT*, days)	3.51	5.78
% of eggs surviving to adults	70	44

hatched when the compound was applied to *T. urticae* adult females (Dimetry *et al.,* 1993). However, Sáenz de Cabezón *et al.* (2002) observed no effect on the fertility when a sublethal concentration of triflumuron (0.75 g L^{-1} of Alsysistin®) to *T. urticae* adults was applied. Therefore, it seems that the concentration is essential to determine the effect level of triflumuron on adult fertility.

Although it is of value to examine how chronic exposure affects the individual components of r_m, the comparison of this parameter values provides insight beyond that available from independent analysis of several life-history parameters (Petitt *et al.,* 1994). In our bioassay, the untreated mites had a significantly higher r_m value than the treated ones. Therefore, the compound reduced the ability to the populations of *T. urticae* to increase.

In conclusion, more semi-field and field testing is required before the acaricidal potential of triflumuron can be considered completely investigated, but the estimation of critical-effect levels from concentration-response relationship for single life cycle variables as mortality, fecundity and fertility performed by Sáenz de Cabezón *et al.* (2002), together with our estimation of the produced sublethal effects could lead to the incorporation of this compound in IPM programs against *T. urticae.*

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