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Sublethal Effects of Spirodiclofen on *Tetranychus urticae* Koch Pre-Ovipositional Females After Different Exposure Times

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SUMMARY

Sublethal effect of spirodiclofen on *Tetranychus urticae* females that survived different exposure times in the pre-ovipositional period was evaluated calculating two parameters - instantaneous rate of increase and net fertility - after six days of reproduction. The females were exposed to four concentrations/doses of the acaricide: 96 mg /L (0.24 µg/cm^2), 48 mg/L (0.12 µg/cm^2), 24 mg/L (0.06 µg/cm^2) and 12 mg./L (0.03 µg/cm^2) for 2, 6 and 24h in a leaf disc bioassay. After 24h exposure to 12 mg/L, instantaneous rate of increase was significantly reduced (0.545; 0.634 in control), while significant reduction in net fertility (20.61; 28.57 in the control) was recorded even after 2h exposure to the same concentration. The effect of all tested concentrations of spirodiclofen on both parameters increased with exposure time. The lowest values of instantaneous rate of increase (0.268) and net fertility (2.58) were recorded after 24h exposure to 96 mg/L. After 24h exposure, the concentration increase from 12 to 24 mg/L significantly reduced both parameters, while a further increase from 24 to 96 mg/L significantly reduced instantaneous rate of increase, but not net-fertility. The results regarding *T. urticae* population management are discussed.

Keywords: Spirodiclofen; *T. urticae*; Instantaneous rate of increase; Net fertility; Sublethal effects

INTRODUCTION

Spirodiclofen, a tetronic acid derivative, has recently been commercialized as an acaricide with a new biochemical mode of action – inhibition of lipid biosynthesis. In field trials worldwide, spirodiclofen has provided excellent control of all relevant phytophagous mite species, including spider mite populations resistant to established acaricides. This acaricide acts against all developmental stages of mites, including eggs, and has a pronounced residual effect (Elbert et al., 2002; Labanowska, 2002; Dekeyser, 2005; Marčić et al., 2005).

In laboratory experiments, spirodiclofen showed high toxicity against eggs and immatures of two-spotted spider mite (*Tetranychus urticae* Koch). Compared

to eggs and juvenile stages, female adults showed a susceptibility several times lower, but fecundity and fertility of the treated survivors were considerably reduced (Nauen et al., 2000; Wachendorff et al.; 2000, 2002; Marčić and Ogurlić, 2006).

Considering its acute toxicity profile and strong residual effect, it is obvious that the recovery of *T. urticae* populations after spirodiclofen treatment depends on the vitality and fertility of females reaching untreated leaf surface. The objective of this study was to evaluate sublethal effects on population growth and reproduction of *T. urticae* females that survived different times of exposure to spirodiclofen in the pre-ovipositional period. The results regarding *T. urticae* population management are discussed.

MATERIAL AND METHODS

Mite culture

The population of *T. urticae* used in these experiments has been maintained on bean plants since March 2004 under long-day conditions (16 h artificial daylight, 25-30°C).

Chemical tested

Spirodiclofen [3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4,5]dec-3-en-4-yl 2,2-dimethylbutyrate], commercial formulation 'Envidor' (suspension concentrate, 240 g/L a.i.), Bayer CropScience, Germany.

Assessment of sublethal effects on pre-ovipositional females

Bean primary leaf discs of 20 mm in diameter, placed on moisturised cotton wool and containing five pre-ovipositional females and two males, obtained from a synchronised mite culture, were treated by air pressure sprayer (0.5 mL of liquid per replication, 100 kPa air pressure, 2.3-2.5 mg wet deposite/cm^2). The commercial formulation of spirodiclofen was serially diluted to four concentrations: 96 mg a.i./L (recommended concentration), 48 mg a.i./L, 24 mg a.i./L and 12 mg a.i./L (concentration causing 100% mortality of eggs and immatures in preliminary tests). Treatment was conducted in 6-10 replications, depending on concentration, and females were exposed to spirodiclofen over three variants of time intervals: 2, 6 and 24 h. After spending 2, 6 or 24 h on treated leaf discs, the survivors were transfered to fresh leaf discs (three females and two males per disc, eight replications). After 24 h, the females were again transferred to new leaf discs and the procedure was repeated six times. The number of females alive, the number of eggs laid (fecundity), the number of eggs hatched (fertility) and the number of female offspring were recorded daily, starting from the transfer of females to the leaf discs in the third time variant (24 h exposure).

Sublethal effects of spirodiclofen were evaluated using two parameters: instantaneous rate of increase and net fertility. The instantaneous rate of increase (r_i) was calculated using the following equation

$$r_i = [\ln (N_f/N_0)]/\Delta t,$$

where N_f was the final number of animals, N_0 was the initial number of animals and Δt was the change in time (number of days the experiment was run). Positive values of r_i indicate a growing population, $r_i = 0$ indicates a stable population, while negative r_i indicates a population in decline. This parameter is a direct measure of population growth that integrates both survivorship and fecundity/fertility (Walthall and Stark, 1997; Stark and Banks, 2003). In this study, N_0 was the initial number of females per replicate ($N_0 = 3$), and N_f was calculated at the end of the sixth day as a total number of eggs, immatures and female adults per replication. The r_i values obtained were subjected to *lsd*-test in ANOVA.

Age-specific curves were given for the following life-table functions: female survival rate (l_x) as the proportion of females in age interval x, and net fertility ($l_x m_x$) as the number of female offspring per female individual in the middle of age interval x (m_x) weighed by female survival rate, where x was the age of females in days (Carey, 1993). Total net fertility for all six days of reproduction was calculated, and the data were subjected to *lsd*-test in ANOVA.

The test was conducted at 26.5 ± 1.5°C, 35-55% RH and 16 h daylight.

RESULTS AND DISCUSSION

Instantaneous rates of increase were significantly lower in all variants of treatment with spirodiclofen than in the untreated control, except partially under the shortest exposure time (Table 1). The effect of all

four concentrations increased with exposure time, but significant difference of 12 and 24 mg/L concentrations, compared to untreated control, was achieved only under the longest exposure time. As concentrations increased, treatment effect after 6 h exposure increased significantly only with 48 mg/L, while further doubling of concentration failed to produce a significant effect. After 24 h exposure, a significant difference from untreated control was recorded even with 12 mg/L, while concentration increase from 48 to 96 mg/L failed to enhance treatment effect.

Instantaneous rate of increase of *T. urticae* in the untreated control in this experiment exceeded 0.350-0.400, the values Stark et al (1997) and Stark and Banken (1999) reported for their untreated control in their seven-day experiments. Lower r_i most probably resulted from the fact that the authors had started their experiments with 8-day old females, thus missing the reproductive maximum. The adaptive strategy of two-spotted spider mite, as a colonizing type of species, is known (Carey, 1982; Sabelis, 1985) to be based on high fecundity/fertility in young females.

Spirodiclofen treatment reduced fecundity and fertility of the surviving *T. urticae* females, and the effect of all four concentrations increased with extending exposure time. Compared with the untreated control, decrease in the number of eggs laid by treated females on the first day of reproduction ranged from 17.4% (2 h exposure) to 96.6% (24 h) (Figure 1A). Fertility reduction was even more evident, ranging from 31.1% to 100% (Figure 1B), as much of the eggs remained unhatched (data not included): 15-71% (2 h exposure), 58-86% (6 h) and 93-100% (24 h), while the untreated control had 5% unhatched eggs.

Wachendorff et al. (2002) found that fecundity of *T. urticae* female adults exposed to tarsal contact (0.44 μg a.i/cm^2) with spirodiclofen-treated surfaces for 2 h was reduced almost to zero one and two days after treatment. In our experiment, the highest dose for females was 0.24 μg a.i/cm^2 (96 mg/L, 2.5 mg wet deposite/cm^2). After 2 h exposure, fecundity decreased on the first day of reproduction by roughly a quarter, and fertility by three quarters. Fecundity was reduced to mere several percent only under 24 h exposure to 24-96 mg/L, i.e. 0.06-0.24 μg a.i/cm^2; reproduction then completely terminated as all eggs laid remained unhatched.

Fertility reduction was also observed to continue over the following two days. On the second day of reproduction, maximum fertility reduction in treated females was 34% (2 h exposure), 53% (6 h exposure) and 75% (24 h exposure), while all concentrations achieved fertility reduction exceeding 10% on the third day only after 24 h exposure.

Besides reducing fecundity and fertility, spirodiclofen also influenced the vitality of surviving females, the effect increasing with duration of exposure (Fig. 2). Decline in the vitality of surviving females, combined with fertility reduction, over the first three days of reproduction was found also to cause a reduction in age-specific fertility, which was most evident after 24 h exposure (Figure 2).

Reduction in age-specific fertility, which was found in all exposure variants over the initial three days of reproduction (Fig. 2), caused a statistically significant reduction in total net fertility (Table 2).

In all variants of spirodiclofen treatment, net-fertility was significantly lower than in untreated control (Table 2). The effect of all four concentrations increased with extended exposure, but statistically significant difference among the three exposure periods was recorded only under two higher concentrations.

Table 1. Instantaneous rate of increase of *T. urticae* after different times of exposure to spirodiclofen (mg/L) in pre-oviposition-al period, and six days of reproduction

Tabela 1. Trenutna stopa rasta *T. urticae* nakon različitog vremena izlaganja spirodiklofenu (mg/L) u pre-ovipozicionom periodu i šestodnevne reprodukcije

Exposure time Vreme izlaganja	Spirodiclofen (mg/L)				
	Untreated Kontrola	12	24	48	96
24 h	0.634 *a*	0.545 *b B*	0.357 *c B*	0.280 *cd C*	0.268 *d C*
6 h		0.583 *a AB*	0.595 *a A*	0.532 *b B*	0.464 *b B*
2 h		0.591 *bc AB*	0.611 *ab A*	0.567 *c B*	0.594 *abc A*
	Untreated Kontrola	0.634 *A*			

The parameters followed by the same letter are not significantly different (P<0.05; lsd-test)
Parametri označeni istim slovom ne razlikuju se značajno (P<0,05; lsd-test)

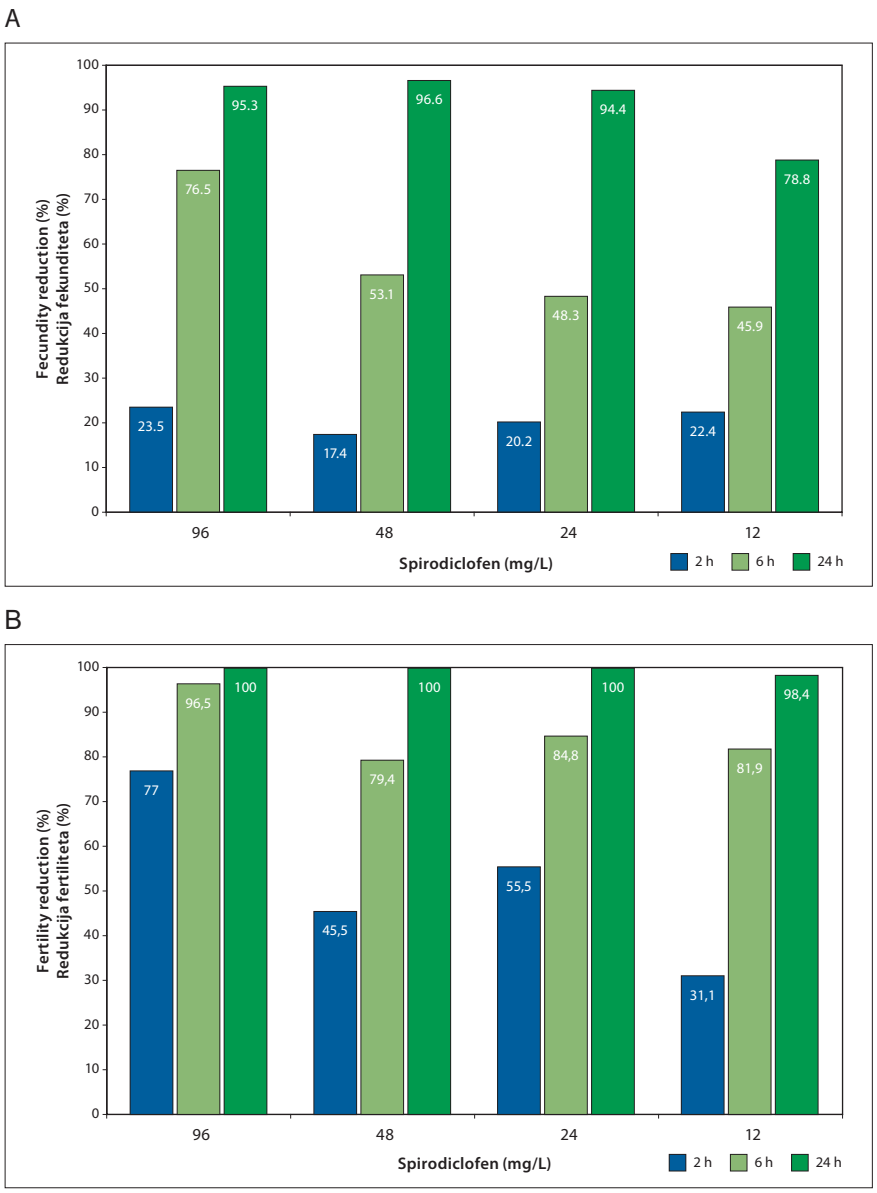

Fig. 1. Reduction in *T. urticae* female fecundity (A) and fertility (B) on the first day of reproduction (%, com pared to untreated females) following exposure to spirodiclofen for 2, 6 and 24 h
Sl. 1. Redukcija fekunditeta (A) i fertiliteta (B) ženki *T. urticae* prvog dana reprodukcije (%, u odnosu na netretirane ženke u kontroli), nakon izlaganja spirodiklofenu 2, 6 i 24 h

After 2 and 6 h exposure, concentration increase did not significantly increase effect; after 24 h exposure, however, the effect increased significantly only as concentration was raised from 12 to 24 mg/L.

Comparing the values of instantaneous rate of increase and net fertility we observed similarities and differences between the two parameters. After 24 h exposure, a significant decrease in the values of both parameters was achieved with the lowest concentration tested, i.e. 12 mg/L. Increase in concentration from 12 to 24 mg/L significantly increased the effect of both parameters, while a further concentration increase from 24 to 96 mg/L significantly reduced instantaneous rate of increase, but not net fertility. The effect was found to increase with exposure time under both parameters, but net fertility values were changing faster as a significant

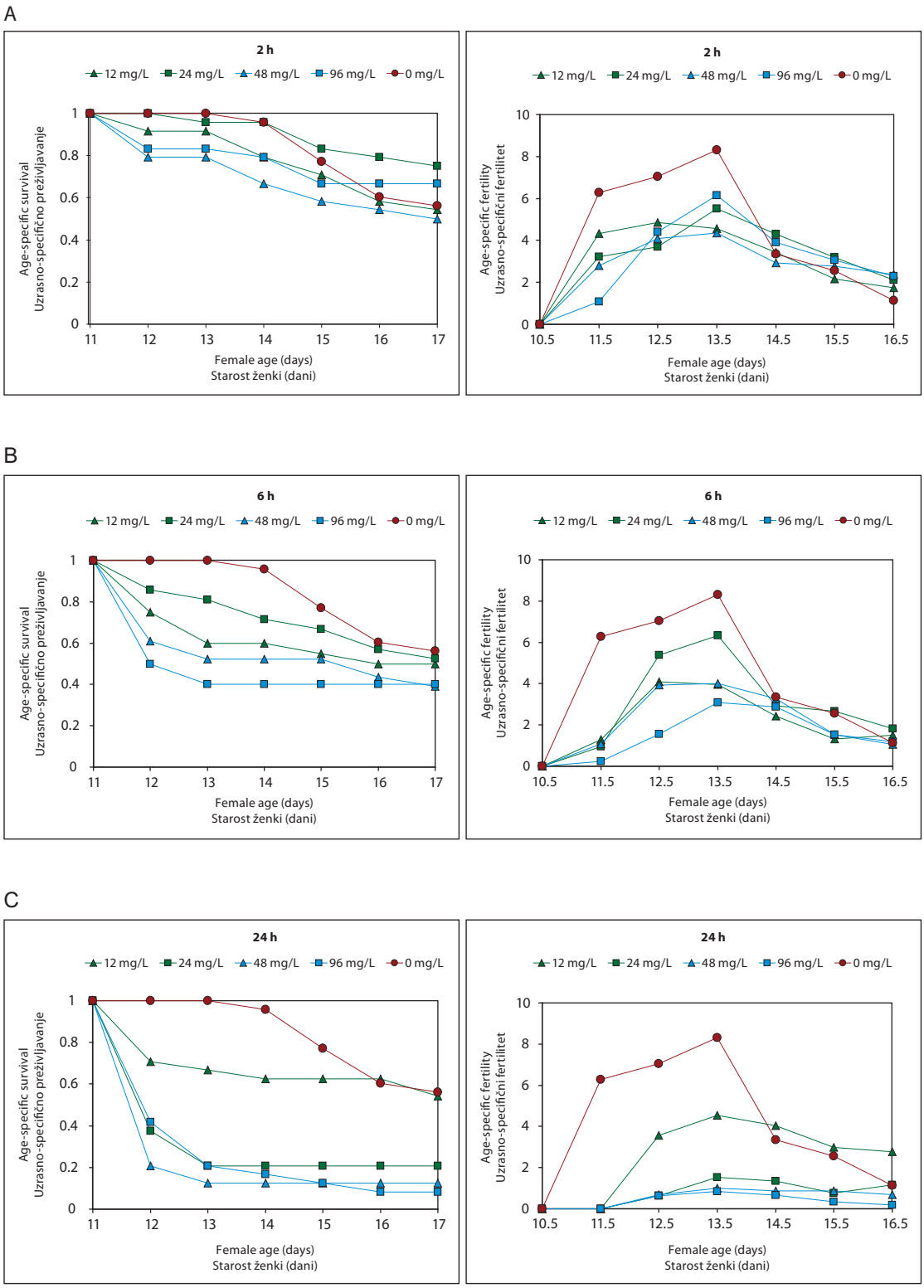

Fig. 2. Age-specific survival and fertility curves for *T. urticae* females exposed to spirodiclofen for 2 (A), 6 (B) and 24 h (C)
Sl. 2. Krive uzrasno-specifičnog preživljavanja i fertiliteta ženki *T. urticae* izloženih spirodiklofenu 2 (A), 6 (B) i 24 h (C)

Table 2. Net fertility of *T. urticae* (number of female offspring per female) after different times of exposure to spirodiclofen (mg/L) in pre-ovipositional period and six days of reproduction
Tabela 2. Neto fertilitet *T. urticae* (broj ženskog potomstva po ženki) nakon različitog vremena izlaganja spirodiklofenu (mg/L) u pre-ovipozicionom periodu i šestodnevne reprodukcije

Exposure time Vreme izlaganja	Spirodiclofen (mg/L)				
	Untreated Kontrola	12	24	48	96
24 h	28.57 *a*	17.93 *b C*	5.42 *c C*	4.12 *c D*	2.58 *c D*
6 h		14.62 *cd C*	20.05 *b B*	14.88 *c C*	10.48 *d C*
2 h		20.61 *b B*	22.04 *b B*	19.28 *b B*	20.91 *b B*
	Untreated Kontrola	28.57 *A*			

The parameters followed by the same letter are not significantly different (P<0.05; lsd-test)
Parametri označeni istim slovom ne razlikuju se značajno (P<0,05; lsd-test)

reduction was achieved already with the 2 h exposure. The differences result, at least partially, from the methods applied to calculate the two parameters: instantaneous rate of increase disregards egg viability and offspring sex ratio, whereas net fertility does not.

In ecotoxicological studies (Wolf et al., 2002; Hardman et al, 2003) the effect of spirodiclofen on beneficial mites was tested only against *Typhlodromus pyri*, a phytoseiid mite well known as an efficient predator of European red mite, *Panonychus ulmi,* in apple orchards. Zacharda and Hluchy (1996) demonstated the successfulness of this phytoseiid predatory mite in controlling *T. urticae* on low-growing crops as well. These studies revealed some adverse effects of spirodiclofen on *T. pyri*. Neumann (2000; loc. cit. Wolf et al., 2002) determined LR_{50} (application rate at which 50% mortality occurs) of *T. pyri* on detached apple leaves as 2.3 g a.i./ha. In our study, the lowest concentration/dose tested at which a significant reduction in net fertility occurs was 12 mg a.i/L, i.e. 0.03 µg a.i/cm^2 = 3 g a.i./ha. This concentration was eight times lower than the recommended concentration and discriminating for eggs and juvenile stages that make up 90% of the stable age distrubition of *T. urticae* natural population. These results give ground to an assessment that management of *T. urticae* could be based on integration of *T. pyri* and reduced rates of spirodiclofen.

REFERENCES

Carey, J.R.: Demography of the two-spotted spider mite, *Tetranychus urticae* Koch. Oecologia, 52: 389-395, 1982.

Carey, J.R.: Applied Demography for Biologists. Oxford University Press, New York, Oxford, UK, 1993.

Dekeyser, M.A.: Acaricide mode of action. Pest Manag. Sci., 61: 103-110, 2005.

Elbert, A., Brück, E., Sone, S. and Toledo, A.: Worldwide uses of the new acaricide Envidor® in perennial crops. Pflanzenschutz-Nachrichten Bayer, 55: 287-304, 2002.

Hardman, J.M., Franklin, J.L., Moreau, D.L. and Bostanian, N.J.: An index for selective toxicity of miticides to phytophagous mites and their predators based on orchard trials. Pest Manag. Sci., 59: 1321-1332, 2003.

Labanowska, B.H.: Efficacy of Envidor 240 SC in the control of the twospotted spider mite (*Tetranychus urticae* Koch) on the black currant plantations in Poland. Proc. of the 8th International *Rubus* and *Ribes* Symposium, East Malling, UK – Acta Hort., 585, 363-367, 2002.

Marčić, D. and Ogurlić, I: Lethal and sublethal effects of spirodiclofen on two-spotted spider mite (Acari: Tetranychidae). Pestic. Phytomed., 21: 137-143, 2006.

Marčić, D., Perić, P., Prijović, M., Ogurlić, I. i Andrić, G.: Efikasnost spirodiklofena u zaštiti jabuke od grinja-paučinara (*Panonychus ulmi*, *Tetranychus urticae*) i kruške od kruškine buve (*Cacopsylla pyri*). Zbornik rezimea VII savetovanja o zaštiti bilja, Soko Banja, 2005, 105.

Nauen, R., Stumpf, N., Elbert, A.: Efficacy of BAJ 2740, a new acaricidal tetronic acid derivative, against tetranychid spider mite species resistant to conventional acaricides. Proc. of the Brighton Crop Protection Conference – Pests and Diseases, 2000, pp. 453-458.

Sabelis, M.W.: Reproductive strategies. In: Spider mites, their biology, natural enemies and control (Helle, W. and Sabelis, M.W., eds.), Vol. 1A, Elsevier, Amsterdam, The Netherlands, 1985, pp. 265-278

Stark, J.D. and Banken, J.A.O.: Importance of population structure at the time of toxicant exposure. Ecotoxicol. Environ. Saf., 42: 282-287, 1999.

Stark, J.D. and Banks, J.E.: Population-level effects of pesticides and other toxicants on arthropods. Ann. Rev. Entomol., 48: 505-519, 2003.

Stark, J.D., Tanigoshi, L., Bounfour, M. and Antonelli A.: Reproductive potential - its influence on the susceptibility of a species to pesticides. Ecotoxicol. Environ. Saf., 37: 273-279, 1997.

Wachendorff, U., Brück, E., Elbert, A., Fischer, R., Nauen, R., Stumpf, N. and Tiemann, R.: BAJ 2740, a novel broad spectrum acaricide. Proc. of the Brighton Crop Protection Conference – Pests and Diseases, 2000, pp. 53-58.

Wachendorff, U., Nauen, R., Schnorbach, H.J., Rauch, N. and Elbert, A.: The biological profile of spirodiclofen (Envidor®) – A new selective tetronic acid acaricide. Pflanzenschutz- Nachrichten Bayer, 55: 149-176, 2002.

Walthall, W.K. and Stark, J.D.: Comparison of two population-level ecotoxicological endpoints: the intrinsic (r_m) and instantaneous (r_i) rates of increase. Environ. Toxicol. Chem., 16: 1068-1073, 1997.

Wolf, G. and Schnorbach, H.J.: Ecobiological profile of the acaricide spirodiclofen. Pflanzenschutz-Nachrichten Bayer, 55: 177-196, 2002.

Zacharda, M. and Hluchy, M.: Biological control of two-spotted spider mite *Tetranychus urticae* on strawberries by the predatory phytoseiid mite *Typhlodromus pyri*. OILB/WPRS Bull., 19: 226-229, 1996.

Subletalni efekti spirodiklofena na *Tetranychus urticae* Koch nakon različitog vremena ekspozicije pre-ovipozicionih ženki

REZIME

Subletalni efekti spirodiklofena na ženke *Tetranychus urticae* koje su preživele različito vreme ekspozicije u pre-ovipozicionom periodu ocenjivani su izračunavanjem dva parametra – trenutne stope rasta i neto-fertiliteta – nakon šest dana reprodukcije. U biotestu na lisnim isečcima ženke su bile izložene 2, 6 i 24h delovanju četiri koncentracije/doze akaricida: 96 mg/L (0.24 µg/cm^2), 48 mg/L (0.12 µg/cm^2), 24 mg/L (0.06 µg/cm^2) i 12 mg/L (0.03 µg/cm^2). Nakon 24-časovne koncentraciji 12 mg/L trenutna stopa rasta bila je značajno redukovana (0.545; 0.634 u kontroli), dok je značajna redukcija neto-fertiliteta (20.61; 28.57 u kontroli) zabeležena već posle 2-časovne ekspozicije istoj koncentraciji. Efekat sve četiri testirane koncentracije spirodiklofena na oba parametra povećavao se produžavanjem ekspozicije. Najniže vrednosti trenutne stope rasta (0.268) i neto-fertiliteta (2.58) zabeležene su posle 24-časovne ekspozicije koncentraciji 96 mg/L. Povećanje koncentracije sa 12 na 24 mg/L značajno je redukovalo oba parametra; dalje povećanje koncentracije sa 24 na 96 mg/L značajno je redukovalo trenutnu stopu rasta, ali ne i neto-fertilitet. Dobijeni rezultati razmatrani su u kontekstu upravljanja populacijama *T. urticae*.

Ključne reči: Spirodiklofen; *T. urticae*; trenutna stopa rasta; neto-fertilitet; subletalni efekti