

Lethal and Sublethal Effects of Spirodiclofen on Two-spotted Spider Mite (Acari: Tetranychidae)

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SUMMARY

Acute toxicity of spiroadiclofen, a tetroneic acid derivative, to developmental stages of two-spotted spider mite, *Tetranychus urticae* Koch, and its sublethal effects on *T. urticae* females that survived treatment at pre-ovipositional period were evaluated in laboratory tests. Acute toxicity tests (0.5 mL of liquid per replication, 100 kPa air pressure, 2.37 ± 0.07 mg wet deposit/cm²) showed a susceptibility to eggs and immatures that was several times higher than to pre-ovipositional females. The following LC-50 values were recorded (in mg/L): 5.1 (eggs); 2.0 (larvae); 2.1 (protonymphs); 3.7 (female deutonymphs) and 36.8 (pre-ovipositional females, after 72 h exposure). The sublethal effects of spiroadiclofen were quantified as net fertility (the number of eggs hatched or the number of female offspring per female) over the initial six days after treatment. Net fertility was significantly reduced over the concentration range of 7.9-96 mg/L. The effect increased with concentration up to 47 mg/L; further increase to 96 mg/L (the recommended concentration) did not significantly increase spiroadiclofen effect. The results regarding *T. urticae* population age/stage structure and control are discussed.

Keywords: Spiroadiclofen; *T. urticae*; Acute toxicity; Sublethal effects

INTRODUCTION

A way of dealing with the resistance of spider mites (Acari: Tetranychidae) is to search for new specific acaricides operating on novel biochemical or physiological targets. Bayer CropScience has discovered and commercialized a tetroneic acid derivative, spiroadiclofen, an acaricide that differs

from others in its biochemical mode of action – inhibition of lipid biosynthesis (Dekeyser, 2005). Spiroadiclofen effectively controls a broad range of phytophagous mites, including spider mite species resistant to conventional acaricides. In laboratory experiments, spiroadiclofen showed high toxicity against eggs and immatures of two-spotted spider mite (*Tetranychus urticae* Koch). Female adults were

also affected and died a few days after direct treatment. Spirodiclofen also reduced fecundity and fertility of females exposed to treated surface (Nauen et al., 2000; Wachendorff et al., 2000 and 2002; Elbert et al., 2002; Labanowska, 2002).

The objective of this study was to evaluate acute toxicity of spirodiclofen to different developmental stages of two-spotted spider mite and its sublethal effects on females that survived treatment at pre-ovipositional period. The results regarding *T. urticae* population age/stage structure and 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.

Toxicity tests

Spirodiclofen acute toxicity to different developmental stages of *T. urticae* was tested in leaf disc bioassay, as described in detail previously (Marčič, 2003). Bean primary leaf discs of 20 mm in diameter, placed on moisturised cotton wool and containing eggs (24 h old), immatures (i.e. larvae, protonymphs, female deutonymphs) or pre-ovipositional females were treated by air pressure sprayer (0.5 mL of liquid per replication, 100 kPa air pressure, 2.3–2.5 mg wet deposit/cm²). According to preliminary tests, the commercial formulation of spirodiclofen was serially diluted in 5–7 concentrations to cover the range of 10–90% mortality. Concentrations were set symmetrically to enable reliable determination of LC-50 (Robertson and Preisler, 1992). The tests had four replications, and sample size was 200–360 individuals. The number of survivors reaching the protonymphal stage (after treatment of eggs) or the adult stage (after treatment

of immatures) was counted. The number of living individuals was counted 72 h after the treatment of pre-ovipositional females. Data were subjected to probit analysis (Finney, 1971). Non-overlapping of 95% confidence limits (CL) was the criterion for significant difference between the stages. The tests were conducted at 25.5 ± 1.5°C, 30–50 % RH and 16 h daylight.

Assessment of sublethal effects on pre-ovipositional females

Five or six pre-ovipositional females and 2–3 males, obtained from synchronised mite culture, were treated per each bean leaf disc in the same way as in the acute toxicity tests. The commercial formulation of spirodiclofen was serially diluted in 10 concentrations, beginning from 96 mg/L (recommended concentration of spirodiclofen). The treatment was conducted in 4–20 replications, depending on concentration. After 24 h, female mortality was assessed, the initial value of female survival rate was calculated and the survivors without visible symptoms of intoxication were transferred to fresh leaf discs (5–9 females per disc, 4 replications). After 48 h, the females were transferred to new leaf discs again and the procedure was repeated twice after that. The number of females alive, the number of eggs laid, the number of eggs hatched and the number of females in the offspring were recorded. Sublethal effects of spirodiclofen were quantified as net fertility *per capita* i.e. the number of eggs hatched or the number of female offspring per female alive at mid-interval of 48 h, weighed by female survival rate, according to Carey (1993). The data were subjected to Duncan's test. The test was conducted at 26 ± 2°C, 35–55 % RH and 16 h daylight.

RESULTS AND DISCUSSION

Table 1 presents the parameters of spirodiclofen toxicity to different developmental stages of *T. urticae*. At the LC-50 level, the most susceptible were larvae and protonymphs, while female deutonymphs showed significantly lower susceptibility. Ovicidal activity of spirodiclofen against 24 h old eggs was significantly lower than against juvenile stages. Compared to eggs and juvenile stages, pre-ovipositional females showed several times lower susceptibility. At the LC-90 level, the same susceptibility

Table 1. Spirodiclofen toxicity parameters for eggs (E), larvae (L), protonymphs (PN), female deutonymphs (DN) and pre-ovipositional females (PF) of *T. urticae***Tabela 1.** Parametri toksičnosti spirodiklofena za jaja (E), larve (L), protonimfe (PN), deutonomife ženke (DN) i pre-ovipozicione ženke (PF) *T. urticae*

	LC-50 (mg/L) (CL 95%)	LC-90 (mg/L) (CL 95%)	b ± SE
E	5.1 (4.6-5.6)	10.5 (9.1-12.8)	4.0 ± 0.4
L	2.0 (1.8-2.1)	3.0 (2.7-3.6)	6.9 ± 1.0
PN	2.1 (1.8-2.3)	4.1 (3.5-5.2)	4.3 ± 0.5
DN	3.7 (3.2-4.2)	9.2 (7.3-13.4)	3.2 ± 0.5
PF	36.8 (31.0-44.6)	88.3 (65.8-161.2)	3.4 ± 0.6

b = Slope of regression line (Nagib regresione linije)

SE = Standard error (Standardna greška)

CL = Confidential limits (Granice poverenja)

relationships remained, only the 95% CL for eggs and deutonymphs overlapped.

Although not being comparable due to different application techniques, these results are analogous to those reported by Wachendorff et al. (2002), who found the pre-ovipositional females to be several times less susceptible than eggs and immatures.

Considering the strong residual activity of spirodiclofen (Wachendorff et al., 2002) and the toxicity parameters obtained in this study, it was obvious that the recovery of *T. urticae* populations depended on the vitality and fertility of females reaching untreated leaf surface. Figure 1 shows the survival rates of *T. urticae* females exposed to spirodiclofen throughout the pre-ovipositional period and the next six days on untreated discs.

For females exposed to the four high concentrations (32.9-96.0 mg/L; mortality after 24 h: 37-60%), transfer to untreated discs did not lead to recovery, so that the surviving percentage of females was a mere 9-16% seven days after treatment, compared to 61% in the control. Recovery was observed in the females exposed to 16.1 and 23.0 mg/L concentrations (mortality after 24 h: 20%), while the survival of the females exposed to the four low concentrations (3.9-11.3 mg/L; mortality after 24 h: 0-8%) were similar from the start to the female survival rate in the control.

Concentration 32.9 mg/L caused a 56% mortality two days after the female transfer to untreated discs; in the acute toxicity test, in which female mortality was assessed after 72 h exposure to treated discs, 95% CL for LC-50 was 31.0-44.6 mg/L (Table 1). A comparison of these results suggests that the initial 24 h exposure period is decisive for spirodiclofen effect.

Apart from survival rate, the number of eggs laid and eggs hatched per female were also examined. The number of eggs laid per female is not presented here as the preliminary tests showed that treated females laid a considerable number of flabby and wrinkled eggs, especially over the initial two day period after the treatment. Wachendorff et al. (2002) also reported that *T. urticae* females treated with sublethal concentrations of spirodiclofen laid non-viable, flabby eggs. The figure 2 shows only the percentage of unhatched eggs.

Over the initial two days after transfer to untreated discs, the four higher concentrations (having the highest effect on the survival rate) produced 42-67% unhatched eggs. The females exposed to 16.1 and 23.0 mg/L concentrations laid 32% and 43% unhatched eggs, while the respective percentage for the four lower concentrations was 12% and 31%. The percentage of unhatched eggs was 9-16% on the fourth and fifth day after the treatment, and 3-11% on the sixth and seventh day after the treatment; an exception to this was 47 mg/L concentration,

Fig. 1. Survival rates of *T. urticae* females after spiroadiclofen treatment (mg/L) at pre-ovipositional period
Sl. 1. Stopa preživljavanja ženki *T. urticae* tretiranih spiroadiklofenom (mg/L) u pre-ovipozicionom periodu

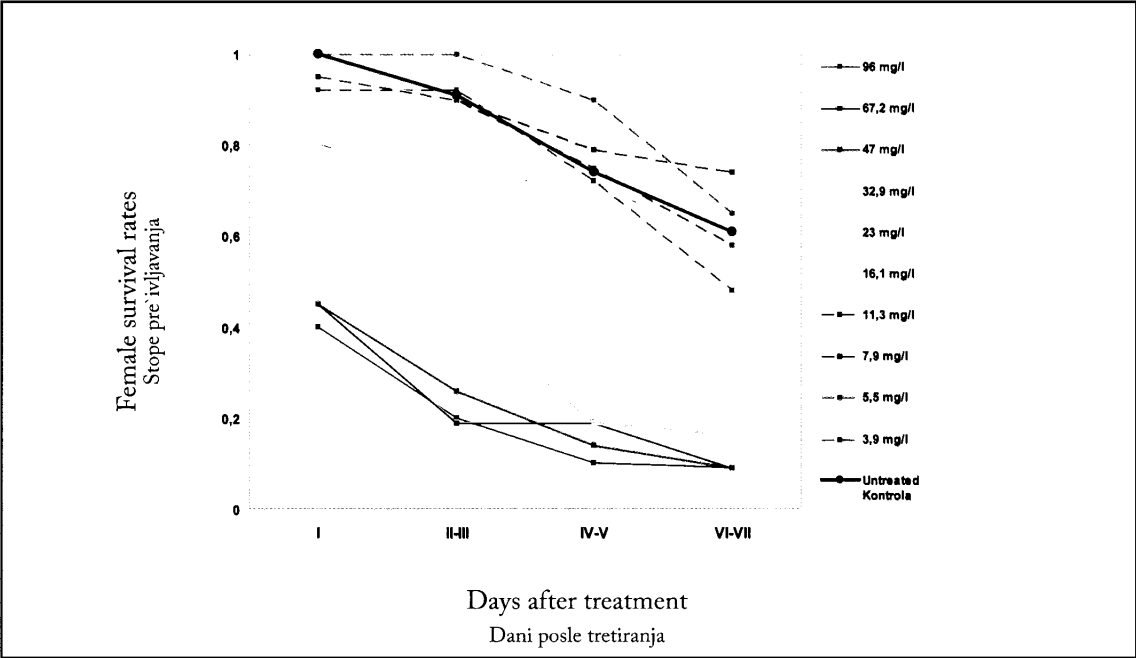


Fig. 2. Percentage of unhatched eggs from *T. urticae* females surviving treatment with spiroadiclofen (mg/L) at pre-ovipositional period (DAT = days after treatment)
Sl. 2. Procenat neispljenih jaja kod ženki *T. urticae* preživelih tretiranje spiroadiklofenom (mg/L) u pre-ovipozicionom periodu (DAT = dani posle tretiranja)

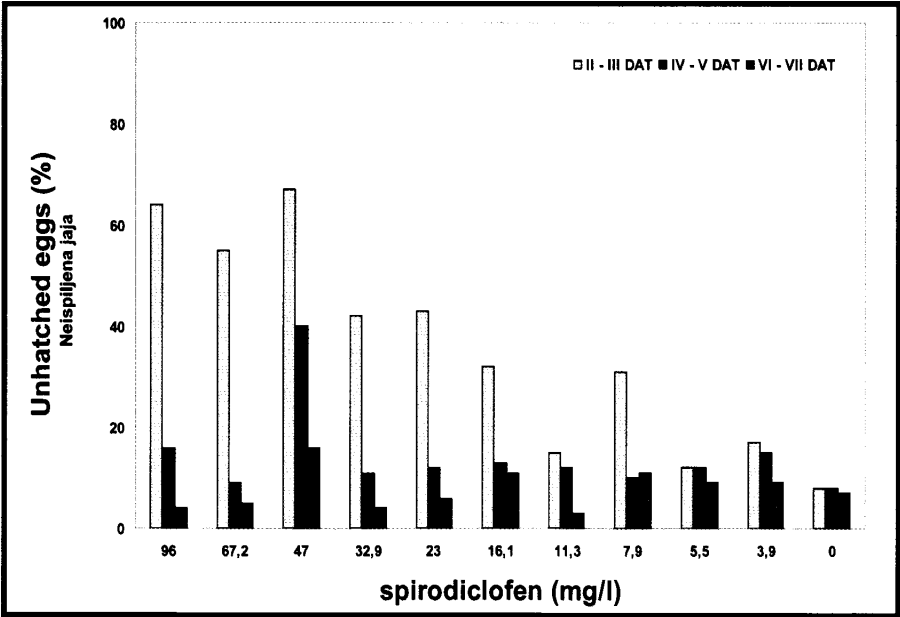


Table 2. Net fertility of *T. urticae* females (number of hatched eggs per female) surviving treatment with spiroticlofen (mg/L) at pre-ovipositional period**Tabela 2.** Neto-fertilitet ženki *T. urticae* (broj ispljenih jaja po ženki) preživelih tretiranje spirotiklofenom (mg/L) u pre-ovipozicionom periodu

mg/L	Days after treatment - Dani posle tretiranja			
	II-III	IV-V	VI-VII	II-VII
96.0	0.2 a	1.1 a	0.8 a	2.1 a
67.2	0.6 a	3.2 ab	2.3 a	6.1 ab
47.0	0.1 a	0.4 a	0.3 a	0.8 a
32.9	1.2 a	4.8 b	1.9 a	7.9 b
23.0	5.4 b	14.4 c	8.9 bc	28.7 c
16.1	6.6 b	14.7 c	7.5 b	28.8 c
11.3	11.3 c	16.2 c	7.2 b	34.7 d
7.9	9.7 c	16.2 c	11.0 cd	36.9 d
5.5	16.0 d	17.8 c	12.4 d	46.2 f
3.9	14.4 d	16.6 c	8.7 bc	39.7 de
Untreated Kotrola	19.0 e	16.1 c	8.8 bc	43.9 ef

The parameters followed by the same letter are not significantly different ($P \leq 0,05$; Duncan's test)

Parametri označeni istim slovom ne razlikuju se značajno ($P \leq 0,05$; Dankanov test)

Table 3. Net fertility of *T. urticae* females (number of female offspring per female) surviving treatment with spiroticlofen (mg/l) at pre-ovipositional period**Tabela 3.** Neto-fertilitet ženki *T. urticae* (broj ženskog potomstva po ženki) preživelih tretiranje spirotiklofenom (mg/L) u pre-ovipozicionom periodu

mg/L	Days after treatment - Dani posle tretiranja			
	II-III	IV-V	VI-VII	II-VII
96.0	0.1 a	0.6 a	0.3 a	1.0 a
67.2	0.4 a	1.7 ab	1.3 a	3.4 ab
47.0	0.1 a	0.2 a	0.2 a	0.5 a
32.9	0.8 a	3.3 b	1.1 a	5.2 b
23.0	3.7 b	8.8 c	4.3 bc	16.8 c
16.1	5.1 bc	9.0 c	3.9 bc	18.0 c
11.3	8.2 d	8.8 c	3.7 bc	20.7 cd
7.9	6.7 cd	9.0 c	4.5 bc	20.2 c
5.5	10.5 e	9.6 c	5.6 c	25.7 e
3.9	10.2 e	10.4 c	3.6 b	24.2 de
Untreated Kotrola	13.1 f	10.0 c	4.7 bc	27.8 e

The parameters followed by the same letter are not significantly different ($P \leq 0.05$; Duncan's test)

Parametri označeni istim slovom ne razlikuju se značajno ($P \leq 0.05$; Dankanov test)

which resulted in 40% and 16% unhatched eggs, respectively. When the percentage of unhatched eggs exceeded that in the control (7-8%) a considerable number of wrinkled eggs was also recorded together with a small number of „normal-looking“ unhatched eggs such as those in the control.

For the sake of easy reference, the gross fertility data (number of hatched eggs per female) were not presented. Compared to the control, the total gross fertility of females exposed to 16.1-96 mg/L concentrations was significantly lower.

Tables 2 and 3 show net fertility, which gives a more realistic view of the recovery potential of a population as the number of eggs hatched or the number of female offspring per female is weighted by female survival rate. Table 2 gives net fertility as the number of hatched eggs per female.

Over the initial two days after the transfer to untreated discs, all concentrations caused a statistically significant reduction in net fertility of the treated females, and the effect was highest for the four higher concentrations. On the fourth and fifth day after the treatment, only those four concentrations reduced net fertility with statistical significance, and the same effect was recorded on the sixth and seventh day after the treatment. Total net fertility was significantly lower than that in the control for all concentrations except the two lowest ones. The highest effect was observed for the four high concentrations, as a result of high mortality of females after transfer to untreated discs, considerable percentage of unhatched eggs and reduced gross fertility. Table 3 shows net fertility as the number of female offspring per female.

The males were not visibly affected after 24 h exposure, which the data reported by Wachendorff et al. (2002) anticipated, and treatment with spiroticlofen did not disturb mating: the surviving females laid eggs and those produced female offspring as well. Total net fertility was here again significantly reduced for all concentrations except the two lowest ones, while the highest effect was recorded for the four higher concentrations. The results show that spiroticlofen treatment basically did not disturb the sex ratio, i.e. the proportion of female offspring was 0.57-0.75 (0.72 in the control).

Acute toxicity tests showed the susceptibility of eggs and immatures to spiroticlofen to be many times higher than that of pre-ovipositional females, and the concentration of 23 mg/L may

be considered discriminative. On the other hand, spiroticlofen significantly reduced net fertility of treated females over the initial six days after being transferred to untreated discs within the concentration range of 7.9-96 mg/L. The effect increased with concentration up to 47 mg/L; further increase to 96 mg/L (concentration recommended for application) did not significantly increase the effect of spiroticlofen. The data may be useful for *T. urticae* control as the natural populations of the pest are normally on the increase, i.e. they are near the stable age distribution of 65% eggs, 25% immatures and 10% adults (Carey, 1982; Sabelis 1985). The results give ground to an assessment that spiroticlofen applied at 23 mg/L or higher rate would eliminate about 90% of the population and reproduction of female survivors would be considerably reduced. As the acaricide, applied at recommended rate, is toxic to predatory mites (Wachendorff et al., 2000; Hardman et al., 2003) and a further concentration rise far beyond the values given within the LC-50 CL for pre-ovipositional females does not significantly increase the effect on survivor reproduction, the management of *T. urticae* populations with spiroticlofen might include application rates lower than the recommended rate, which would allow the activity of predatory mites and reduce selection pressure.

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Letalni i subletalni efekti spirodiklofena na običnog paučinara (Acari: Tetranychidae)

REZIME

Akutna toksičnost spirodiklofena, derivata tetrionske kiseline, za razvojne stadijume običnog paučinara, *Tetranychus urticae* Koch, i subletalni efekti na ženke *T. urticae* koje su preživele tretiranje ovim akaricidom u preovipozicionom periodu, ispitivani su u laboratorijskom ogledu. Testovi akutne toksičnosti (0.5 mL tečnosti po ponavljanju, pritisak vazduha 100 kPa; 2.37 ± 0.07 mg/cm² svežeg depozita) pokazali su višestruko veću osetljivost jaja i juvenilnih stadijuma na spirodiklofen, u odnosu na preovipozicione ženke. Dobijene su sledeće LC-50 vrednosti (u mg/L): 5.1 (jaja); 2.0 (larve); 2.1 (protonimfe); 3.7 (ženke deutonomife) i 36.8 (preovipozicione ženke, ekspozicija 72 h). Subletalni efekti spirodiklofena kvantifikovani su kao neto fertilitet (broj ispljenih jaja, odnosno broj ženskog potomstva po ženki) tokom prvih šest dana nakon tretiranja. Koncentracije u rasponu od 7.9 mg/L do 96 mg/L značajno su redukovale neto fertilitet. Sa povećanjem koncentracija povećavao se i efekat, sve do 47 mg/L; dalje povećanje do 96 mg/L (koncentracija preporučena za primenu) nije značajno povećalo efekat spirodiklofena. Dobijeni rezultati razmatrani su u kontekstu uzrasne strukture i suzbijanja prirodnih populacija *T. urticae*.

Ključne reči: Spirodiklofen; *T. urticae*; akutna toksičnost; subletalni efekti