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The Effect of Spiromesifen on the Reproductive Potential of *Tetranychus urticae* Koch (Acari: Tetranychidae)

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SUMMARY

Laboratory bioassays were conducted to evaluate the effects of spiromesifen on the fecundity, fertility and population growth of two-spotted spider mite (*Tetranychus urticae* Koch) after treatment of pre-ovipositing females with five acaricide concentrations: 180 mg/l (maximum recommended concentration for use in glasshouses against spider mites), 18 mg/l, 1.8 mg/l and 0.18 mg/l (the last one was discriminative for eggs and immatures in preliminary studies, i.e. produced 100% mortality of those stages) and 0.018 mg/l. After 24h exposure, the percentages of females surviving treatment without visible symptoms of poisoning were 50% (180 mg/l), 45% (18 mg/l), 51% (1.8 mg/l), 74% (0.18 mg/l), 96% (0.018 mg/l) and 98% (0 mg/l). Over the first four days after treatment, the females that survived 180 mg/l and 18 mg/l laid no eggs. The total number of eggs laid after treatment with these two concentrations was reduced to less than 2% against the control by the end of the trial. The females that survived 1.8 mg/l laid 50% less eggs, compared to the control, while the number of eggs laid by the females treated with 0.18 mg/l and 0.018 mg/l were 19% and 4% lower, respectively. Over the initial four days, egg hatch rates in treatments were 73-87%, and 92-93% in the control. Significant statistical differences between gross fecundity (*FCg*) and gross fertility (*FTg*) values in the control and treatments were detected for females surviving 180 mg/l, 18 mg/l and 1.8 mg/l. On the other hand, only the net fertility (*FTn*) value of females treated with 0.018 mg/l showed no statistically significant difference from the control value. Treatments with 180 mg/l and 18 mg/l significantly reduced the instantaneous rate of increase (r_i) 6, 8 and 10 days after treatment, compared to the control. The negative r_i values in those treatments indicated a declining population. Sublethal effects of spiromesifen and its impact on *T. urticae* management are discussed.

Keywords: *Tetranychus urticae*; Spiromesifen; Sublethal effects

INTRODUCTION

Exploitation of new compounds acting on novel biochemical and physiological targets has been an important aspect of acaricide research. Spirodiclofen and spiromesifen, tetronic acid derivatives, have recently been introduced as acaricides with novel mode of action (inhibition of acetyl-CoA-carboxylase), highly effective against all relevant phytophagous mite species, including mite populations resistant to other acaricides (Dekeyser, 2005; Bretschneider et al., 2007). In laboratory bioassays with two-spotted spider mite, *Tetranychus urticae*, spirodiclofen showed high acute toxicity to eggs and immature stages. Its activity on female adults was slower, but fecundity and fertility of the treated individuals were significantly reduced (Wachendorff et al., 2002; Marčić, 2007; Van Pottelberge et al., 2009).

Biological profile of spiromesifen against *T. urticae* is clearly comparable with that described for spirodiclofen: high toxicity to eggs and juvenile stages, slower activity on female adults with strong influence on fecundity/fertility, the same symptomology of poisoning, lack of visible impact on male adults even after treatments with high concentrations, and a pronounced residual effect (Nauen et al., 2005). From this point of view, it is obvious that the recovery of two-spotted spider mite populations from treatment with spiromesifen mostly depends on the reproductive potential of adult females reaching untreated leaf surface.

Dispersal and colonization are important elements in the biology of *T. urticae*, contributing to its persistance in natural and agroecosystems. Mite age is among the factors influencing dispersal: mated pre-ovipositional adult females of two-spotted spider mites are most likely to exhibit dispersal behavior (Mitchell, 1973; Li and Margolies, 1993; Yano, 2008). As the adaptive strategy of this species is based on a high reproductive potential of young and fertilized female dispersers (Sabelis 1985), in this work we evaluated the effects of spiromesifen on the fecundity, fertility and instantaneous rate of increase of *T. urticae* after treatment at the pre-ovipositing stage. The mites were treated with five spiromesifen concentrations, the highest being the maximum recommended rate in EU for use in glasshouses against spider mites.

For chemical pest control to be genuinely rational, it is necessary to evaluate the overall impact of a pesticide, i.e. to assess its effects on life history traits of the survivors, apart from acute mortality estimates. Moreover, lethal and sublethal effects could be integrated as a population-level response using population growth rates as endpoints (Robertson and Worner, 1990; Stark et al., 1997; Stark and Banks, 2003). This approach has been used in bioassays with spider mites (Marčić, 2003, 2005, 2007; Teodoro et al., 2005; Kim et al., 2006 ; Li et al., 2006; Marčić and Ogurlić, 2006). The objective of this study was to expand our knowledge on sublethal effects of spiromesifen on *T. urticae* and evaluate these effects in terms of improving the pest's population management.

MATERIAL AND METHODS

Population tested

A population of *T. urticae* formed from individuals collected from a ruderal weed flora habitat in Belgrade environs has been reared on bean plants in a climate chamber (16/8h of light/dark photoperiod, 25-30°C) since March 2004. To establish a synchronous mite culture, adult females were selected from the population and placed on bean leaf discs (Ø 30 mm), positioned upon moisturised cotton wool in Petri dishes (100 discs, 5 females per disc, 24h oviposition). The culture was monitored until the stage of quiescent female deutonymphs and adult males that were selected for the bioassay.

Chemical tested

Spiromesifen, commercial formulation Oberon® (suspension concentrate, 240 g a.i./l, Bayer CropScience, Germany).

Assessment of sublethal effects

Sublethal effects of spiromesifen on *T. urticae* were evaluated as effects on its fecundity, fertility and population growth. The assays were carried out in a climate chamber under 27±2°C, 30-50% RH and 16h daylight. From the synchronous culture quiescent female deutonymphs and adult males were transferred to new leaf discs (Ø 30 mm, 10-15 quiescent female deutonymphs and 5 adult males per disc). The acaricide suspended in distilled water was applied by air pressure sprayer (100 kPa, 0.5 ml liquid) to the discs. The following concentrations of spiromesifen were used: 180 mg/l (maximum recommended concentration in EU for use in glasshouses against spider mites), 18 mg/l,

1.8 mg/l and 0.18 mg/l (this concentration was discriminative for eggs and immatures in preliminary studies, i.e. produced 100% mortality of these stages) and 0.018 mg/l. Control individuals were sprayed with distilled water alone. Mites were treated several hours after the emergence of adult females from teleiochrysalises and the surviving females were removed from discs after 24h. The assays were conducted in four replicates, with two leaf discs per replicate.

The isolated female survivors (10 per replicate) were placed individually on untreated leaf discs (Ø 30 mm). Over the following 10 days, the females were transferred every 48h to new discs and the number of females alive (*Fs*) and eggs laid were simultaneously monitored. Female survival rates were calculated as *Fs*/10. Hatch rate was defined as the percentage of eggs hatched from a total number of eggs laid. *Gross fecundity* (the number of eggs laid per female; *FCg*), *gross fertility* (the number of eggs hatched per female, *FTg*), and *net fertility* (gross fertility weighted by female survival rates; *FTn*) were defined and calculated according to Carey (1993). Fecundity/fertility data were summed, square-root transformed and analysed by one-way ANOVA with the means separated by Tukey-test ($p<0.05$). Untransformed means are presented in this paper.

The effect of spiromesifen on population growth was measured by *the instantaneous rate of increase* (ri) calculated by the following equation:

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

where N_0 was the initial number of individuals (i.e. 10 adult females per replicate), Nt was the number of individuals at the end of t^{th} day (i.e. the number of adult females alive, eggs laid and hatched larvae), and Δt was the number of days elapsed between the start of the bioassay and the end of t^{th} day. Positive r_i values indicate a growing population, negative

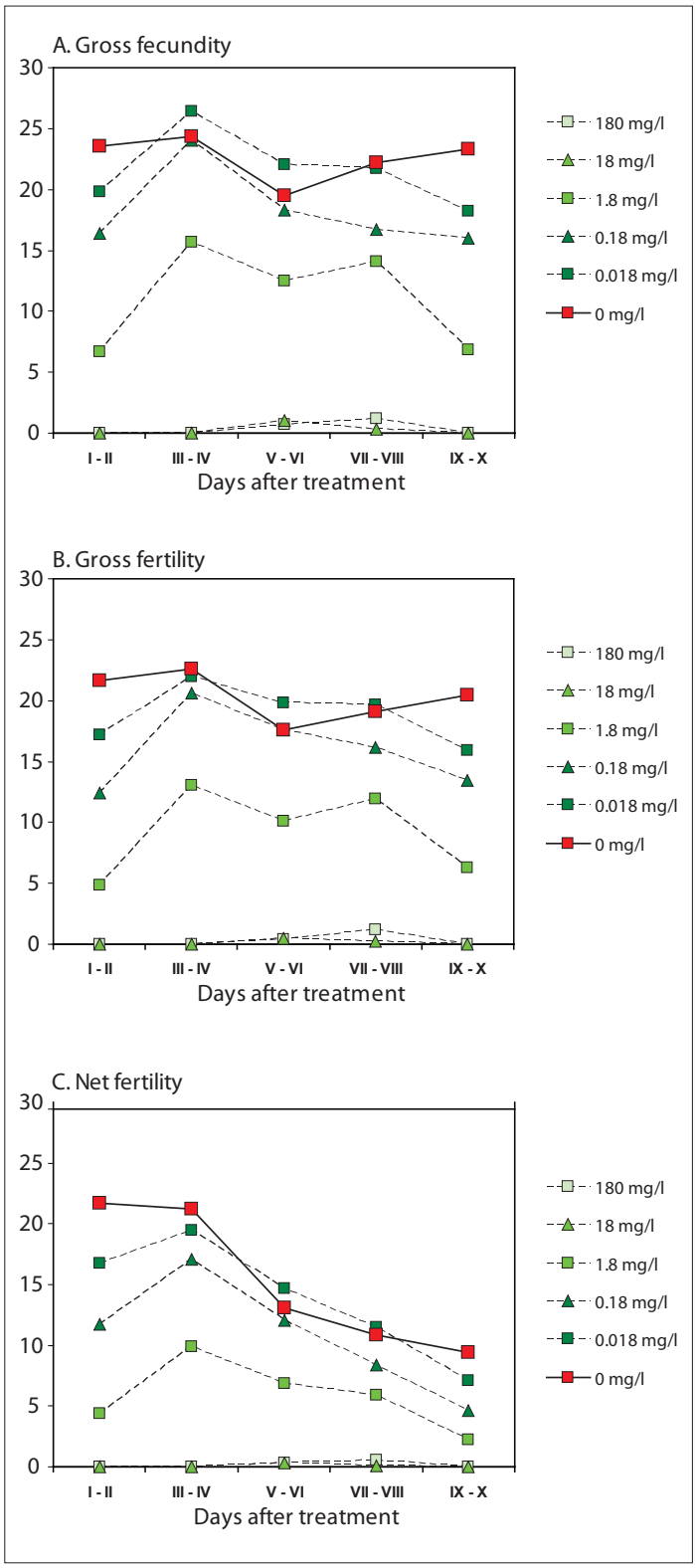

Figure 1. Fecundity and fertility of *T. urticae* females surviving treatment with spiromesifen (mg/l) at pre-ovipositional period

r_i values indicate a population in decline and $r_i = 0$ indicates a stable population (Stark and Banks, 2003). Calculated r_i values were analysed by one-way ANOVA, the means were separated by Tukey-test ($p<0.05$).

RESULTS

After 24h of exposure, the percentages of females that survived treatment without visible symptoms of poisoning were 50% (180 mg/l), 45% (18 mg/l), 51% (1.8 mg/l), 74% (0.18 mg/l), 96% (0.018 mg/l) and 98% (0 mg/l). Figure 1 shows fecundity and fertility curves of the surviving females over 10 days following treatment.

Over the first four days after treatment, the females that survived 180 mg/l and 18 mg/l laid no eggs, while those surviving treatment with 1.8 mg/l laid 53% less eggs than control females. The females that survived 0.18 mg/l and 0.018 mg/l laid 30% and 16% less eggs, respectively, in the initial two days after treatment, compared to the control, but the number of eggs laid reached those in the control by the third and fourth day. By the end of the trial, the total number of eggs laid in treatments with the two highest concentrations was below 2 eggs/female, while the females surviving 1.8 mg/l, 0.18 mg/l and 0.018 mg/l laid 7-14, 16-18 and 18-22 eggs/female/48h, respectively, and untreated females laid 20-23 eggs/female/48h (Figure 1A). Gross fertility curves had a trend similar to gross fecundity curves, but the difference between eggs hatched in the control and treatment in the initial four days was slightly higher than it was for eggs laid as the hatch rate was 73-87% in the treatments, and 92-93% in the control (Figure 1B). Due to lowered survival rates of treated females, especially of those surviving 180 mg/l, 18 mg/l and 1.8 mg/l, their net fertility curves dropped below the curve of females in control (Figure 1C).

Table 1 shows fecundity and fertility as sums over a period of 10 days after treatment. Significant statistical differences between gross fecundity (*FCg*) and gross fertility (*FTg*) values in the control and treatment were detected for females surviving treatment with 180 mg/l, 18 mg/l and 1.8 mg/l, and the values in treatments with the two top concentrations also differed from treatment with 1.8 mg/l. On the other hand, only the net fertility (*FTn*) value of females treated with the lowest concentration showed no statistically significant difference from the control value.

The treatments with 180 mg/l and 18 mg/l significantly reduced the instantaneous rate of increase 6, 8 and 10 days after treatment, compared to the control (Table 2). Negative r_i values obtained in these treatments indicated a declining population.

DISCUSSION

Spiromesifen applied at 180 mg/l, 18 mg/l and 1.8 mg/l strongly affected fecundity and fertility of *T. urticae* female survivors. Treatment with 0.18 mg/l, the concentration discriminative for eggs and immatures, significantly affected only net fertility values. The most prominent effects were in treatments with 180 mg/l and 18 mg/l, where fecundity and fertility of the surviving females were reduced to less than 2% against the control, causing a population decline. These concentrations produced about 50% mortality after treatment of pre-ovipositing females. After the treatment with 1.8 mg/l, fecundity and fertility were reduced by 50-62%, but the instantaneous rate of increase was not significantly different from the control.

Nauen et al. (2005) found that the fecundity of two-spotted spider mite females directly treated on bean leaves was strongly reduced 48h after treatment with spiromesifen concentrations ranging between 0.064 and 40 mg/l: the lowest concentration halved the number of eggs laid, while the highest brought fecundity almost to null. In our study, no eggs were laid over the first four days after treatment with 180 mg/l and 18 mg/l, and fecundity was toppled over 98% by the end of the trial. The treated females that laid eggs in the first four days had lowered hatch rates. A similar pattern of sublethal activity of spirodiclofen had been previously reported (Marčić, 2007; Van Pottelberge et al., 2009).

The data obtained in our study indicated that potential *T. urticae* dispersers from the leaf surface treated with spiromesifen would be significantly affected even by a concentration 100 times lower than the recommended one. Sublethal effects of spiromesifen were more pronounced than those of spirodiclofen, whose lowest concentration causing significant sublethal effect was 16 times lower than the recommended (Marčić, 2007). Considering that eggs and immatures account for around 90% of the stable age distribution of two-spotted spider mite (Carey 1982; Sabelis 1985), it is obvious that relatively low concentrations of spiromesifen could eliminate a considerable part of *T. urticae* popu-

Table 1. Life history traits of *T. urticae* within 10 days after treatment with spiromesifen (mg/l) at pre-ovipositional period

mg/l	*FCg*	*FTg*	*FTn*
180	1.92 (± 1.18) c	1.62 (± 1.05) c	0.80 (± 0.59) d
18	1.32 (± 0.96) c	0.73 (± 0.50) c	0.41 (± 0.24) d
1.8	55.91 (± 6.97) b	46.13 (± 6.03) b	29.29 (± 1.84) c
0.18	91.40 (± 2.38) a	80.24 (± 3.06) a	53.90 (± 5.38) b
0.018	108.25 (± 2.68) a	94.58 (± 3.14) a	69.58 (± 2.10) ab
0.0	112.91 (± 3.64) a	101.45 (± 2.10) a	76.26 (± 6.12) a

Values (± SEM) in columns followed by different letters differ significantly (Tukey-test, $p<0.05$)
FCg = gross fecundity
FTg = gross fertility
FTn = net fertility

Table 2. The instantaneous rate of increase (day^{-1}) of *T. urticae* after treatment of pre-ovipositing females with spiromesifen (mg/l)

mg/l	6 DAT	8 DAT	10 DAT
180	0.005 (± 0.044) b	-0.043 (± 0.080) b	-0.075 (± 0.074) b
18	-0.068 (± 0.098) b	-0.055 (± 0.077) b	-0.098 (± 0.059) b
1.8	0.539 (± 0.011) a	0.425 (± 0.010) a	0.342 (± 0.007) a
0.18	0.629 (± 0.023) a	0.488 (± 0.014) a	0.400 (± 0.010) a
0.018	0.673 (± 0.013) a	0.522 (± 0.010) a	0.428 (± 0.006) a
0.0	0.681 (± 0.010) a	0.529 (± 0.009) a	0.436 (± 0.008) a

Values (± SEM) in columns followed by different letters differ significantly (Tukey-test, $p<0.05$)
DAT = days after treatment

lations. The strongly reduced reproductive capacity of young females as potential dispersers found in our assay with spiromesifen indicates slow recovery of two-spotted spider mite populations.

Spider mites are among major pests of glasshouse crops worldwide. Their control is hindered by rapid evolution of resistance to many chemical classes of pesticides, which has created a permanent need to develop and introduce compounds with novel modes of action. Spiromesifen, as an inhibitor of acetyl-CoA-carboxylase, is just such a product. In order to prevent/delay resistance development and save the product's longevity, it has been recommended to follow the general principles of pest resistance management, including monitoring aimed to detect early signs of resistance (Nauen and Konanz, 2005). Studying sublethal effects of spirodiclofen on the reproduction of a susceptible and a resistant strain of *T. urticae*, Van Pottelberge et al. (2009) found a lack of inhibition of reproduction in resistant mites and stressed that assessment of sublethal effects should be included in monitoring of resistance development. The same logic applies to spiromesifen, which has demonstrated a similar pattern of sublethal activity on two-spotted spider mites. On the other hand, knowing the sublethal effects closely could be seen as a starting point for further research aimed at integrating chemical treatment with biological control agents.

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Efekat spiromesifena na reproduktivni potencijal *Tetranychus urticae* Koch (Acari: Tetranychidae)

REZIME

U laboratorijskom ogledu ispitivani su efekti spiromesifena na fekunditet, fertilitet i populacioni rast običnog paučinara (*Tetranychus urticae* Koch) nakon tretiranja pre-ovipozicionih ženki sledećim koncentracijama akaricida: 180 mg/l (maksimalna preporučena koncentracija za primenu protiv grinja-paučinara u zaštićenom prostoru), 18 mg/l, 1,8 mg/l, 0,18 mg/l (koncentracija diskriminativna za jaja i juvenilne stadijume, tj. koncentracija koja je izazvala 100% smrtnost ovih stadijuma u preliminarnim testovima) i 0,018 mg/l. Nakon 24-časovne ekspozicije, procenat ženki koje su preživele tretman bez vidljivih simptoma iznosio je 50% (180 mg/l), 45% (18 mg/l), 51% (1,8 mg/l), 74% (0,18 mg/l), 96% (0,018 mg/l) i 98% (0 mg/l). U prva četiri dana nakon tretiranja, ženke koje su preživele 180 mg/l i 18 mg/l nisu polagale jaja. Do kraja ogleda, ukupan broj jaja koja su položile ove ženke bio je redukovan na manje od 2%, u poređenju sa kontrolom. Ženke koje su preživele 1,8 mg/l položile su za 50% manje jaja, u poređenju sa kontrolom, dok je broj jaja koja su položile ženke preživele tretiranje koncentracijama 0,18 mg/l i 0,018 mg/l bio manji za 19%, odnosno 4%. U prva četiri dana, stopa piljenja jaja u tretmanu iznosila je 73-87%, a u kontroli 92-93%. Statistički značajne razlike između bruto-fekunditeta (*FCg*) i bruto-fertiliteta (*FTg*) u kontroli i tretmanu utvrđene su za ženke koje su preživele 180 mg/l, 18 mg/l i 1,8 mg/l. S druge strane, jedino se kod ženki tretiranih koncentraciojom 0,018 mg/l neto-fertilitet (*FTn*) nije statistički značajno razlikovao od kontrole. Tretiranje koncentracijama 180 mg/l i 18 mg/l značajno je redukovalo trenutnu stopu rasta (r_i) 6, 8 i 10 dana nakon ekspozicije. Negativne r_i vrednosti dobijene u ovim tretmanima pokazuju da je populacija u opadanju. U radu je razmatran značaj subletalnih efekata spiromesifena za upravljanje populacijama *T. urticae*.

Ključne reči: *Tetranychus urticae*; spiromesifen; subletalni efekti