

## EFFECTS OF SPIRODICLOFEN ON THE REPRODUCTIVE POTENTIAL OF TWO-SPOTTED SPIDER MITE (ACARI: TETRANYCHIDAE) OVIPOSITING FEMALES

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**Abstract** — A laboratory bioassay was conducted to evaluate the effects of spirodiclofen on the survival and reproduction of young and mated females of two-spotted spider mite (*Tetranychus urticae* Koch). The females were sprayed with a series of acaricide concentrations (96, 48, 24, 12, and 6 mg/l) 24-30 h after adult emergence, i.e., at the age most likely to exhibit dispersal behavior and close to their reproductive maximum. The proportions of *T. urticae* females that survived treatment without symptoms of poisoning were concentration-dependent, ranging between 0.41 and 0.88 (0.96 in the control). With the exception of females that survived 6 mg/l, fecundity of the treated female mites was strongly affected during the exposure, compared to the control. The mean daily fecundity (EL) and mean daily fertility (EH) of surviving females, transferred daily to new leaf disks over the following five days, significantly decreased as spirodiclofen concentrations increased. In treatments with 6 mg/l and 12 mg/l, only the latter concentration significantly reduced both EL and EH, compared to the control. In females that survived 24 mg/l and 48 mg/l, these life history parameters were reduced by over 90%, while treatment with 96 mg/l completely terminated egg-laying. The treated females lived for a significantly shorter time than untreated ones, with the exception of females that survived 6 mg/l. Compared to the control females, gross fecundity (GL) and gross fertility (GH) of the treated females were strongly reduced on the first and second day; from the third day onward, females treated with the lowest concentrations achieved marked recovery, their GL and GH going even above the values in the control. However, net fecundity (NL) and net fertility (NH) of all treated females decreased considerably throughout the trial, indicating that survival rates of these females were lower, compared to the control. Calculated as total sums of gross and net daily schedules within five days, fecundity and fertility significantly decreased as spirodiclofen concentration increased. The two lowest concentrations failed to achieve a significant reduction of GL, while GH, NL, and NH were significantly lower than control values starting with the females treated with 6 mg/L. A high percentage of unhatched eggs, especially during the initial two days after treatment (35-100%), further contributed to the significant reduction in fertility of the females treated with spirodiclofen. All concentrations of spirodiclofen significantly reduced the instantaneous rate of increase. Regression analysis showed a linear population decline with increased acaricide concentrations ( $y = 1.13 - 0.24x$ ;  $R^2 = 0.91$ ,  $p < 0.05$ ).

**Key words:** *Tetranychus urticae*, spirodiclofen, survival, reproduction, population growth

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### INTRODUCTION

Of all spider mites (Acari: Prostigmata, Tetranychidae) that are primarily plant-feeding mites, the two-spotted spider mite (*Tetranychus urticae* Koch) is the most polyphagous species, being reported on about 1200 host plants. Two-spotted spider mite is a colonizing species with a short life cycle, rapid population growth, and natural populations usually in the increasing phase (Sabelis, 1985; Bolland et al., 1998; Walter and Proctor, 1999). Although this

cosmopolitan spider mite species occurs on virtually every agricultural and ornamental crop, including a diversity of initially unfavorable hosts (Gould, 1979; Fry, 1989; Agrawal, 2000), it is the most important common pest of greenhouse plants in temperate zones in economic terms (Zhang, 2003; Petanović, 2004). Synthetic pesticides (acaricides) have been widely used to control *T. urticae* and other spider mites. However, the pest has a remarkable intrinsic potential for rapid evolution of resistance to pes-

ticides (acaricides) owing to its polyphagous feeding, short life cycle, a large number of generations, arrhenotokous reproduction, and other biological characteristics (Croft and van de Baan, 1988, Head and Savinelli, 2008). Exposed to heavy selection pressure, especially in greenhouses, *T. urticae* populations have developed resistance to many chemical classes of acaricides throughout the world: among the top 10 resistant arthropod pests, two-spotted spider mite ranks first (Whalon et al., 2008). This situation has created a permanent need to develop and introduce acaricides with novel biochemical modes of action. On the other hand, rational and/or reduced pesticide use has been emphasized as an essential element of any resistance management program (Hoy, 1998). For chemical pest control to be genuinely rational, it is necessary to evaluate the overall impact of a pesticide (acaricide), i.e., to assess its sublethal effects on life history traits of the survivors, apart from measuring acute mortality. Moreover, these sublethal effects could be integrated as a population-level response using population growth rates as endpoints (Robertson and Worner, 1990; Walthall and Stark, 1997; Stark and Banks, 2003). This approach has already been used in bioassays with *T. urticae* and various acaricidal compounds (Stark et al., 1997; Stark and Banken, 1999; Marčić, 2003, 2005; Kim et al., 2006).

Spirodiclofen is an acaricide with a new mode of action, inhibition of lipid biosynthesis, and a unique symptomatology of poisoning. Laboratory acute toxicity bioassays have revealed considerably higher spirodiclofen susceptibility in pre-adult life stages (eggs, larvae, protonymphs, and deutonymphs) of *T. urticae* than in the adult stage of females, as the acaricidal effect is slower against the latter stage. After treatment with concentrations several times higher than those producing 100% mortality of eggs and immatures, female adults live on for another few days, but their fertility becomes fully or partially reduced (Wachendorff et al., 2002; Marčić and Ogurlić, 2006; Cheon et al., 2007; Marčić, 2007). Spirodiclofen residues on plant leaves continue to affect spider mites as long as several weeks after the application (Wachendorff et al., 2002; Cheon et al., 2007). From this point of view, it is obvious that the

recovery of two-spotted spider mite populations from acaricide treatments mostly depends on the vitality and reproductive capacity of adult females reaching untreated leaf surface.

This study focuses on sublethal effects of spirodiclofen on two-spotted spider mite females that survived treatment with the acaricide as young and ovipositing adults. Dispersal and colonization are important elements in the biology of *T. urticae*, contributing to its persistence in natural and artificial ecosystems. Mite age is among the factors influencing dispersal: young (1-2 days old) and mated adult females of two-spotted spider mites are most likely to exhibit dispersal behavior (Li and Margolies, 1993, 1994; Yano, 2008). As the adaptive strategy of *T. urticae* is based on high reproductive potential of young dispersing adult females (Carey, 1982; Sabelis, 1985), in this work we evaluated the effects of spirodiclofen on the survival and reproductive capacity of the dominant dispersers in this colonizing species, treated at the age close to their reproductive maximum.

## MATERIALS AND METHODS

### *Population tested*

A population of *T. urticae* formed from individuals collected from a ruderal weed flora habitat in the environs of Belgrade has been reared on bean plants in a climate chamber under long-day conditions (16 h artificial daylight, 25-30°C) since March 2004. Its susceptibility to spirodiclofen was tested and confirmed previously (Marčić and Ogurlić, 2006). To establish synchronous culture, mites were collected from this population and placed on bean leaf disks, Ø 20 mm, positioned upon moisturized cotton wool in Petri dishes (100 disks, 5 quiescent female deutonymphs and 5 adult males per disk). The culture was monitored and examined every 4-6 h; 24-30 h after adult emergence, mated females that laid eggs 6-8 h previously were selected for the bioassay.

### *Chemical tested*

Spirodiclofen; a tetrionic acid derivative; commercial formulation 'Envidor' (suspension concentration, 240 g/l a.i.), Bayer CropScience.

### Assessment of spirodiclofen effects

The effects of spirodiclofen on *T. urticae* females were evaluated as effects on life history traits and population growth. The acaricide suspended in distilled water was applied to bean leaf disks (Ø 20 mm, placed upon moisturized cotton wool in Petri dishes) by an air-pressure sprayer (100 kPa, 0.5 mL liquid), producing an aqueous deposit of 4.00-4.25 mg/cm<sup>2</sup>. The assays were conducted in a climate chamber under conditions of 28 ± 2°C, 40-50% RH, and 16 h daylight.

Groups of five adult females, obtained from the culture, were placed on each leaf disk and sprayed with one of five serial concentrations of spirodiclofen tested: 96 mg/l (recommended for use against *T. urticae* in Europe), 48 mg/l, 24 mg/l, 12 mg/l, and 6 mg/l (discriminative for eggs and immatures, i.e., causing 100% mortality of these stages in preliminary studies). Depending on concentration, 7-17 leaf disks were sprayed with the acaricide; control individuals were sprayed with distilled water on five disks. After 24-h exposure, the females unable to move after being prodded by a fine brush were scored as dead. Among the survivors, females able to move, but showing symptoms of poisoning (bigger size, swollenness, sticky remains on the ovipositor) were separated and counted. The proportion of the remaining females without symptoms was assessed ( $P_{FS}$ ), six cohorts with 25 females were established, and the females were placed individually on untreated leaf disks. The number of eggs laid per female during the exposure was calculated as well. Over the following five days, the females were transferred daily to new disks and the number of females alive ( $F_s$ ) and the number of eggs laid were simultaneously monitored. Female survival rates were calculated as  $(F_s/25) \times P_{FS}$ .

The effects of spirodiclofen were estimated by calculating the following life history traits: *mean daily fecundity* ( $E_L$ ) and *mean daily fertility* ( $E_H$ ), defined as the mean number of eggs laid/hatched daily per female alive at midpoint of the interval during five days or less, i.e., until a female's death; *average longevity* ( $L$ ), defined as the mean number of days that a female lived after treatment; *gross fecun-*

*dity* ( $G_L$ ) and *gross fertility* ( $G_H$ ), i.e., the number of eggs laid/hatched per female alive at midpoint of the interval; *net fecundity* ( $N_L$ ) and *net fertility* ( $N_H$ ), i.e., gross fertility weighted by female survival rates. The parameters were calculated daily and within five days. The number of eggs hatched was determined at the end of the fourth day after oviposition. The terms "gross", "net", "fecundity", and "fertility" were defined and calculated according to Carey (1993).

The effect of spirodiclofen on population growth was measured by *the instantaneous rate of increase* ( $r_i$ ), calculated by 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  $\Delta t$  was the number of days elapsed between the start and the end of the bioassay. Positive  $r_i$  values indicate a growing population, negative  $r_i$  values indicate a population in decline, and  $r_i = 0$  indicates a stable population (Stark et al., 1997; Stark and Banks, 2003). In this study,  $N_0$  was the initial number of females in cohorts (25) and  $N_f$  was obtained at the end of the fifth day ( $\Delta t = 5$ ) as the total number of female adults alive, immatures hatched, and eggs laid.

### Statistical analysis

The variances of the calculated parameters ( $E_L$ ,  $E_H$ ,  $L$ ,  $G_H$ ,  $G_L$ ,  $N_H$ ,  $N_L$ ,  $r_i$ ) were estimated by the jackknife resampling method based on repeated calculation of the parameters (Meyer et al., 1986; Maia et al., 2000). From data sets with  $n = 25$  observations (i.e., mites), each observation was left out in turn and the values were calculated on the remaining  $n-1$  observations. Pseudo-values of the parameter ( $Pv$ ) were calculated by the following equation:

$$Pv = n Pt - (n - 1)Pi$$

where  $Pt$  was a parameter calculated from the total sample ( $n$ ) and  $Pi$  was a parameter calculated from  $n-1$  observations. After calculation of all  $n$  pseudo-values, the mean, variance, and standard error were obtained; the limits of 95% confidence intervals were calculated by multiplication of the standard error by the  $t$  value from Student's distribution for

$n-1$  degrees of freedom. Non-overlapping of the confidence intervals was the criterion for significant differences between the values of a parameter.

Regression analysis was done to evaluate the effect of spiroadiclofen concentrations on the instantaneous rate of increase.

## RESULTS

Females of *T. urticae* were treated with spiroadiclofen on leaf disks 24-30 h after emergence from the teleiochrysalis. After 24 h of exposure to the acaricide (Table 1), the proportion of dead females ranged between 0.44 and 0.06, depending on concentration (0.04 in the control). The proportion of females showing symptoms of poisoning (swollenness, sticky remains on ovipositors) was similar (0.15-0.20) in all treatments, except in the treatment with the lowest concentration; those females were alive, but unable to lay eggs. The proportion of females that survived treatment without symptoms of poisoning was concentration-dependent and ranged between 0.41-0.88 (0.96 in the control). With the exception of females that survived 6 mg/l, the fecundity of treated females was strongly affected during exposure, compared to the control. However, even females treated with the highest concentration were able to lay more than one egg.

The average daily production of eggs by females

**Table 1.** Survival and fecundity of *T. urticae* females treated with spiroadiclofen (mg/l) 24-30 h after adult emergence. Values followed by different letters differ significantly (based on 95% CLs). Abbreviations: PFD = proportion of females dead after 24 h. PFSM = proportion of females alive after 24 h with symptoms of poisoning. PFS = proportion of females alive after 24 h without symptoms of poisoning. E = number of eggs laid per female during 24-h exposure.

mg/l	P <sub>FD</sub>	P <sub>F<sub>SM</sub></sub>	P <sub>F<sub>S</sub></sub>	E
96	0.44	0.15	0.41	1.3 c
48	0.36	0.17	0.47	1.8 c
24	0.29	0.20	0.51	3.1 b
12	0.18	0.16	0.66	4.4 b
6	0.06	0.06	0.88	8.8 a
0	0.04	0.00	0.96	8.7 a

that survived treatment without symptoms of poisoning and were transferred daily to new leaf disks over the following five days, is shown in Table 2. The mean daily fecundity ( $E_L$ ) and mean daily fertility ( $E_H$ ) significantly decreased as concentrations of spiroadiclofen increased in a concentration-dependent manner (Table 2). Treatment with 96 mg/l completely terminated egg-laying. For the females that survived 48 mg/l and 24 mg/l, the reduction of  $E_L$  was 94% and 92%, respectively, while these concentrations reduced  $E_H$  by 96%. In treatments with 12 mg/l and 6 mg/l, only the former significantly reduced both  $E_L$  and  $E_H$ . Compared to the control, treated females had a considerably greater difference between the average number of laid and hatched eggs. Moreover, these females lived for a significantly shorter time than untreated ones, with the exception of females that survived 6 mg/l.

Figures 1 and 2 show daily fecundity and fertility curves for the untreated females and those that survived treatment with 6, 12, 24, and 48 mg/l over five days (females treated with 96 mg/l laid no eggs during the trial). Compared to the control, gross fecundity ( $G_L$ ) and gross fertility ( $G_H$ ) of the treated females were strongly reduced on the first and second day, and the females treated with 48 mg/l laid no eggs on the first day following treatment. From the third day onward, females treated with the lowest concentrations recovered visibly, having  $G_L$  and

**Table 2.** Mean daily fecundity (EL), mean daily fertility (EH), and average longevity (L) of *T. urticae* females that survived treatment with spiroadiclofen (mg/l) and were transferred to new leaf disks daily over a period of five days. Values followed by different letters differ significantly (based on 95% CLs).

mg/L	E <sub>L</sub>	E <sub>H</sub>	L (days)
96	0.0 d	0.0 d	2.4 cd
48	0.7 c	0.4 c	2.1 d
24	0.9 c	0.4 c	2.3 d
12	6.2 b	4.0 b	3.4 bc
6	9.8 a	5.5 b	4.2 ab
0	11.3 a	10.4 a	4.4 a

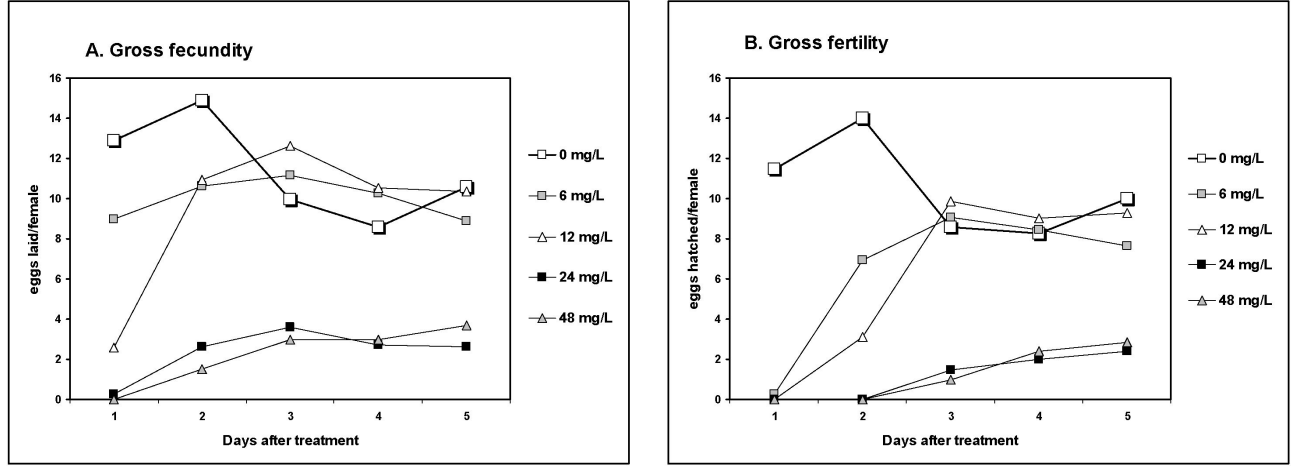


Fig. 1.

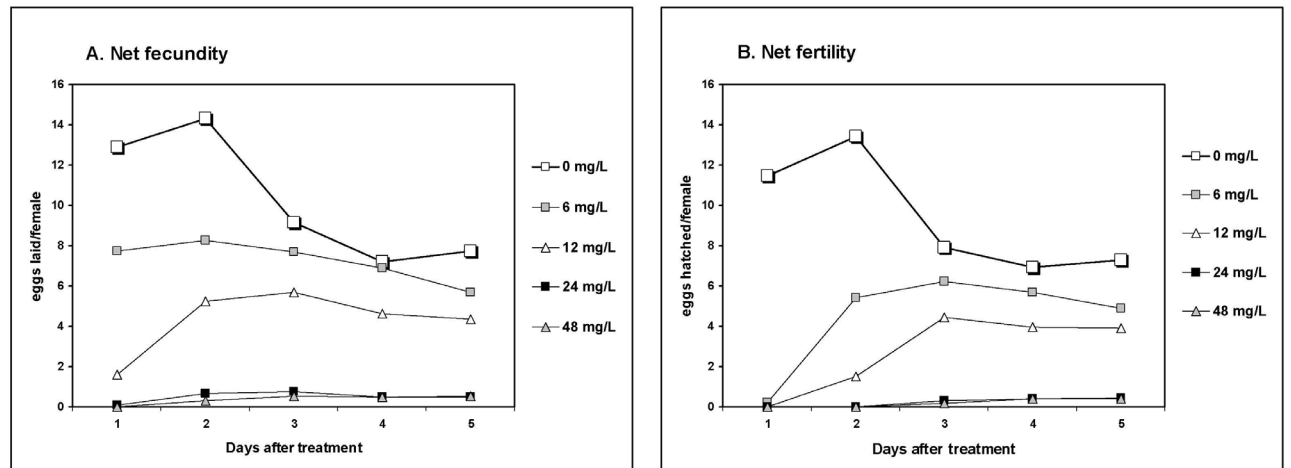


Fig. 2.

**Table 3.** Gross fecundity (GL), gross fertility (GH), net fecundity (NL), and net fertility (NH) of *T. urticae* females within five days after treatment with spirodiclofen (mg/l). Values followed by different letters differ significantly (based on 95% CLs).

mg/L	G <sub>L</sub>	G <sub>H</sub>	N <sub>L</sub>	N <sub>H</sub>
96	0.0 c	0.0 d	0.0 d	0.0 c
48	11.2 b	6.2 c	3.7 c	1.9 c
24	11.8 b	5.8 c	4.5 c	2.0 c
12	47.0 a	31.3 b	30.3 b	19.4 b
6	49.9 a	32.4 b	41.7 b	25.7 b
0	56.9 a	52.3 a	51.3 a	47.0 a

**Table 4.** Instantaneous rate of increase ( $r_i$ ) of *T. urticae* females surviving treatment with spirodiclofen (mg/l). Values followed by different letters differ significantly (based on 95% CLs).

mg/L	$r_i$
96	-0.322 d
48	0.242 c
24	0.245 c
12	0.630 b
6	0.682 b
0	0.773 a

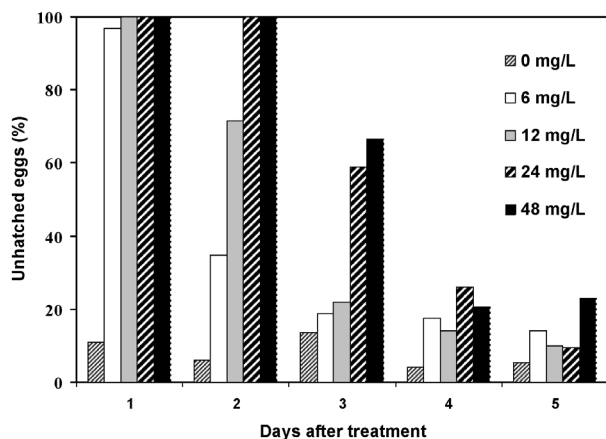


Fig. 3.

$G_H$  even above control values (Fig. 1). However, net fecundity ( $N_L$ ) and net fertility ( $N_H$ ) of all treated females were considerably lowered throughout the trial, indicating that survival rates of these females were lower, compared to the control (Fig. 2). Such an effect was expected, considering that the treated females lived for a shorter time.

Calculated as total sums of gross and net daily schedules within five days, fecundity and fertility significantly decreased as the concentration of spiroadiclofen increased (Table 3), gross fecundity being less affected than the other parameters. The two lowest concentrations caused no significant reduction in  $G_L$ , while  $G_H$ ,  $N_L$ , and  $N_H$  were significantly lower than control values for all treated females.

The fertility of females treated with spiroadiclofen was further lowered by a high percentage of unhatched eggs, especially on the first two days following treatment (Fig. 2). That percentage was 97-100% on the first day, 35-100% on the second, while there were 11% and 6% unhatched eggs in the control. From the third day onward, treated females gradually began to recover.

All concentrations of spiroadiclofen significantly reduced the instantaneous rate of increase ( $r_i$ ) in a concentration-dependent manner. The negative  $r_i$  value obtained for the highest concentration indicated a population heading for extinction (Table 4). Regression analysis showed a linear population decline with increasing acaricide concentrations ( $y = 1.13 - 0.24x$ ;  $R^2 = 0.91$ ,  $p < 0.05$ ).

## DISCUSSION

Coinciding with data reported by Wachendorff et al. (2002), the activity of spiroadiclofen against adult females of two-spotted spider mite was slow in our study: after 24 h of exposure, the highest concentration (96 mg/l) achieved only 44% mortality. Similarly, Cheon et al. (2007) sprayed leaf disks with *T. urticae* female adults with 90 mg/l of spiroadiclofen and observed 18% mortality after 24 h. Besides dead individuals, females with symptoms of poisoning unable to lay eggs were also observed among the survivors in all treatments. The symptoms of poisoning observed in our study were similar to those described by Wachendorff et al. (2002).

Although treatment with 96 mg/l (the concentration recommended against *T. urticae*) caused mortality below 50%, reproduction of the females that survived the treatment completely terminated. The percentages of females surviving 48 mg/l and 24 mg/L without symptoms of poisoning were 47 and 51%, respectively, but their mean daily fertility was reduced to almost zero. On the other hand, only 6% of the females died within 24 h after treatment with 6 mg/l, but the mean daily fertility of the survivors was practically halved. Gross and net daily schedules of reproduction revealed that reduced survival rates and large numbers of unhatched eggs considerably contributed to the net fertility reduction in treated females. As a measure of population growth, the instantaneous rate of increase integrates survival and fertility data. The treatment with 6 mg/l was sufficient for a statistically significant reduction in population growth, while the  $r_i$  values of females surviving treatment with 24 mg/l were three times lower than control values. Treatment with 96 mg/l resulted in a population heading for extinction.

The results obtained in our study confirmed that assessment of sublethal effects on the mite's biology, quantified as an impact on life history traits and population growth, is important for evaluating overall effects of a pesticide (acaricide). Previously, several authors conducted their studies with various acaricidal compounds using this approach to evaluate acaricide toxicity (Stark et al., 1997; Stark and Banken, 1999; Teodoro et al., 2005; Kim et al.,

2006) and emphasized its advantage against acute mortality estimates. Recently, van Pottelberge et al. (2009) investigated the reproduction capacities of spirodiclofen-resistant and spirodiclofen-susceptible strains of *T. urticae*. In contrast to the susceptible strain, fecundity and fertility of the resistant mites were not affected by treatment with the acaricide. The authors stressed that detection of resistance to spirodiclofen should not be limited to mortality bioassays, but should be combined with studies on reproduction of survivors.

Sublethal effects should be considered also in a process of developing control/management strategies for the two-spotted spider mite. Spirodiclofen strongly reduced the survival and reproduction of *T. urticae* females treated at the age close to their reproductive maximum. Compared to females treated at the beginning of the pre-ovipositional period (Marčić, 2007), ovipositing females were less affected, especially by the two lowest concentrations; however, these concentrations still demonstrated a considerable inhibitory effect. Field and greenhouse founder populations of two-spotted spider mite are mostly composed of young females (Fernandez et al., 2008; Yano, 2008). In a situation when survivors reach untreated leaf surface and start to lay eggs, their potential for population growth and recovery would be considerably lowered, even after treatment with relatively low concentrations. By reducing the number of treatments, selection pressure would decrease on mite populations and slow down the evolution of resistance to acaricides. On the other hand, use of acaricide treatments at rates/concentrations below those recommended would be justified on condition that it creates conditions for an integration of chemical treatment and release of biological control agents, such as phytoseiid predatory mites (Acari: Phytoseiidae), a method that has already been found worthwhile as a sustainable control strategy (Herron et al., 1993; Lilly and Campbell, 1999; Rhodes et al., 2006; Cheon et al., 2007). In the case of spirodiclofen, further laboratory, greenhouse, and field data are needed to support this strategy.

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**ЕФЕКТИ СПИРОДИКЛОФЕНА НА РЕПРОДУКТИВНИ ПОТЕНЦИЈАЛ ОВИПОЗИЦИОНИХ ЖЕНКИ ОБИЧНЕ ПАУЧИНАСТЕ ГРИЊЕ (ACARI: TETRANYCHIDAE)**

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Ефекти спироциклофена на преживљавање и репродукцију младих и оплођених женки обичне паучинасте гриње (*Tetranychus urticae* Koch) испитивани су у лабораторијском огледу. Женке су прскане серијом концентрација акарицида (96, 48, 24, 12 и 6 мг/л) 24-30 х након појаве адулта, тј. у узрасту блиском репродуктивном максимуму, када је највећа вероватноћа за дисперзију. Пропорција женки *T. urticae* које су преживеле третман без симптома тровања зависила је од концентрације и кретала се између 0.41 и 0.88 (0.96 у контроли). Са изузетком женки које су преживеле 6 мг/л, фекундитет третираних женки био је знатно умањен током експозиције, у поређењу са контролом. Средњи дневни фекундитет ( $E_L$ ) и средњи дневни фертилитет ( $E_H$ ) преживелих женки, пребациваних дневно на нове лисне исечке током пет дана, значајно су се смањивали како се концентрација повећавала. У третманима са 6 мг/л и 12 мг/л, само је ова друга концентрација значајно редуковала и  $E_L$  и  $E_H$ , у односу са контролу. Код женки које су преживеле 24 мг/л или 48 мг/л ови животни параметри били су редуковани за преко 90 %, док је третман са 96 мг/л потпуно прекинуо полагање јаја. Третиране женке живе су значајно краће од нетретираних, осим женки третираних са 6

мг/л. У поређењу са контролом, бруто фекундитет ( $G_L$ ) и бруто фертилитет ( $G_H$ ) третираних женки били су знатно смањени првог и другог дана после третирања; од трећег дана надаље, женке третиране нижим концентрацијама видно су се опоравиле а њихове  $G_L$  и  $G_H$  вредности биле су чак и изнад одговарајућих вредности у контроли. Међутим, нето фекундитет ( $N_L$ ) и нето фертилитет ( $N_H$ ) свих третираних женки били су знатно умањени за све време трајања огледа, што указује на умањење стопа преживљавања. Израчунати као укупна сума дневних бруто и нето вредности, фекундитет и фертилитет третираних женки значајно су опадали са повећањем концентрација спироциклофена. Две најниже концентрације нису оствариле значајну редукацију  $G_L$ , док су  $G_H$ ,  $N_L$  и  $N_H$  били значајно нижи код свих третираних женки. Висок проценат неиспиљених јаја, посебно у прва два дана после третирања (35-100 %) додатно је допринео значајној редукацији укупног фертилитета женки третираних спироциклофеном. Све концентрације акарицида значајно су редуковале тренутну стопу раста популације. Регресиона анализа показала је линеарно опадање популације у зависности од повећања концентрације спироциклофена ( $y = 1.13 - 0.24x$ ;  $R^2 = 0.91$ ,  $p < 0.05$ ).