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The Effects of Spirodiclofen on Reproduction of Two-spotted Spider Mite (*Tetranychus urticae* Koch)

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SUMMARY

Laboratory bioassay was conducted to evaluate the effects of spirodiclofen on the survival, fecundity and fertility of two-spotted spider mite (*Tetranychus urticae* Koch) females treated as 3-days old adults with a series of acaricide concentrations starting with the concentration discriminative for eggs and immatures. After a 24 h exposure, the proportion of females that survived treatment was 0.86 (6 mg/L), 0.71 (12 mg/L), 0.54 (24 mg/L), 0.50 (48 mg/L) and 0.44 (96 mg/L). Over the following five days, the survival rates of females treated with 6 mg/L and 12 mg/L were considerably below the survival rate of untreated females, but they still remained above the survival rates of females treated with other concentrations. Total fecundity/fertility significantly decreased as concentrations of spirodiclofen increased. The highest concentration, 96 mg/L, completely terminated egg-laying, while only two and three females of those surviving the respective concentrations of 48 mg/L and 24 mg/L laid viable eggs. On the other hand, 60% and 84% of female survivors of treatments with the respective concentrations of 12 mg/L and 6 mg/L laid viable eggs; total fertility of these females was reduced by 58.6 and 45.2%, respectively. On the first day after treatment, the females treated with 24 mg/L, 12 mg/L and 6 mg/L laid eggs; viable eggs were laid only by the latter group and the percentage of hatching was barely 3.1% (89% in control). On the second day, the females treated with 48 mg/L also began to lay eggs, but viable eggs were laid only by females treated with 12 mg/L and 6 mg/L (the respective percentages of hatching were 28.5% and 65.3%; 93.9% in control). From the third day onward, viable eggs were laid also by females treated with 48 mg/L, and the difference in hatchability was considerably smaller or disappeared completely. Compared to control, gross fecundity was significantly reduced by all concentrations on the first day only, and gross fertility on the first two days of trial. No significant difference in gross fecundity/fertility was observed further on until the end of the trial between untreated females and those treated with 6 mg/L and 12 mg/L. However, all concentrations significantly reduced net fecundity/fertility throughout the trial, which indicates a considerable impact of the decreased female survival rate on overall reduction in net fertility, especially from the third day onward. Sublethal effects of spirodiclofen and its impact on *T. urticae* management are discussed.

Keywords: *Tetranychus urticae*; Spirodiclofen; Reproduction

INTRODUCTION

Spirodiclofen is an acaricide with a novel mode of action (inhibition of lipid synthesis), highly effective against all relevant phytophagous mite species, including mite populations resistant to other acaricides (Nauen et al., 2000; Elbert et al., 2002; Dekeyser 2005). In laboratory bioassays with two-spotted spider mite, *Tetranychus urticae*, spirodiclofen demonstrated high acute toxicity to eggs and immatures, while female adults were less susceptible. Its activity against females was slower: after direct treatment, most females died after 3-5 days, but fecundity and fertility of the surviving individuals were significantly reduced (Wachendorff et al., 2002; Marčić and Ogurlić, 2006a, 2006b; Cheon et al., 2007). Considering that eggs and immatures account for around 90% of the stable age distribution in *T. urticae* as a colonizing species (Carey, 1982; Sabelis, 1985) it is obvious that a relatively low concentration of spirodiclofen would eliminate a considerable part of such two-spotted spider mite population. Integrated control of *T. urticae* using acaricides and phytoseiid mites has been suggested (Easterbrook, 1992; Trumble and Morse, 1993; Lilley and Campbell, 1999; White, 2004) as a most effective management strategy for this pest. However, in laboratory and field trials with spirodiclofen some adverse effects on predatory mites were observed (Wolf and Schnorbach, 2002; Hardman et al., 2003; Reis et al., 2006; Welty, 2006; Cheon et al., 2007). Successful control can be achieved by releasing predators in conjunction with reduced rates of acaricides (Herron et al., 1993; Rhodes et al., 2006).

Previously, we investigated (Marčić, 2007) sublethal effects of spirodiclofen on the life history and life-table parameters of two-spotted spider mite females treated the pre-ovipositional stage with a series of acaricide concentrations, beginning with concentration discriminative for eggs and immatures and up to recommended concentration. The former significantly affected fertility and population growth rates of the females that survived treatment, while a concentration four times lower than the recommended stopped reproduction. This study focuses on effects of spirodiclofen on the survival, fecundity and fertility of *T. urticae* females treated as 3-days old adults with the same series of acaricide concentrations. The objective was to evaluate these effects from the aspect of stable age distribution, in terms of a possible integration of chemical (spirodiclofen applied at rates below those recommended) and biological (phytoseiid mites release) control measure in the management of two-spotted spider mite populations.

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 under long-day conditions (16 h of artificial daylight, 25-30^{0}C) since March 2004.

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.), obtained from Bayer CropScience, Germany, was used.

Assessment of the effects of spirodiclofen

The effects of spirodiclofen on *T. urticae* females were evaluated as effects on survival, fecundity and fertility. The acaricide suspended in distilled water was applied to bean leaf discs (20 mm in diameter, placed upon moisturised cotton wool in Petri dishes) by air pressure sprayer (100 kPa, 0.5 mL liquid per replicate), producing aqueous deposit of 4-4.25 mg/cm^2. The assays were conducted in a climate chamber under 28.5 ± 2^{0}C, 35-55% RH and 16 h daylight.

A group of five adult females, 2.5-3.5 days old, obtained from synchronous cultures, was placed on each leaf disc and sprayed with one of five serial concentrations of spirodiclofen tested: 6 mg/L (in preliminary studies, it was the lowest acute toxic concentration to females after 24h exposure; this concentration was also discriminative for eggs and immatures, i.e. produced 100% mortality of these stages), 12 mg/L, 24 mg/L, 48 mg/L and 96 mg/L (recommended for use against *T. urticae* in Europe; Elbert et al., 2002). Depending on concentration, the acaricide was applied in 5-17 replicates. Control individuals were sprayed with distilled water in five replications.

After 24h exposure, the eggs laid during exposure were counted, the proportion of females that survived treatment (P_{FS}) was assessed, and cohorts of 25 females

without visible symptoms of poisoning were selected from the survivors and placed individually on untreated leaf discs. Over the following five days, the females were transferred daily to new discs and the number of females alive (*Fs*) and eggs laid were simultaneously monitored. **Female survival rates** were calculated as $(Fs/25)\times P_{FS}$. The number of eggs hatched was determined five days after oviposition. **Total fecundity/fertility** was defined as the total mean number of eggs laid/hatched per female within five days (or less, i.e. until a female's death). **Gross fecundity/fertility** (the mean number of eggs laid/hatched daily per female alive) and **net fecundity/fertility** (gross fecundity/fertility weighted by female survival rates) were defined and calculated according to Carey (1993).

The fecundity/fertility data were transformed by $\sqrt{n}$ or $\sqrt{(n + 0.5)}$ and the means separated by *t*-test ($p<0.05$). Untransformed means are presented in this paper.

RESULTS AND DISCUSSION

After a 24 h exposure to spirodiclofen, the proportion of *T. urticae* females that survived treatment was 0.86 (6 mg/L), 0.71 (12 mg/L), 0.54 (24 mg/L), 0.50 (48 mg/L) and 0.44 (96 mg/L); there were no dead females in control. During exposure, treated females laid significantly fewer eggs than untreated individuals, except females treated with the lowest concentration. There was a statistically significant fecundity reduction in females threated with the two highest concentrations, compared to females treated with 24 mg/L i 12 mg/L, which indicates that the effect decreased as concentrations increased (Table 1). Over the following five days, the survival rates of females treated with 6 mg/L and 12 mg/L were considerably below the survival rate of females in control, but they still remained above the survival rates of females treated with other concentrations (Figure 1). Previously, we observed a similar effect of spirodiclofen on female viability after treatment in the pre-ovipostional stage (Marčić, 2007).

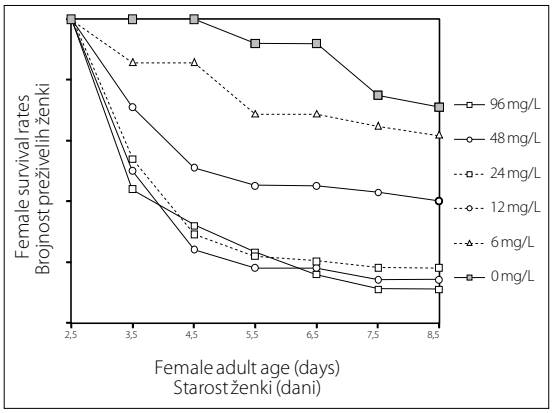

Figure 1. Survival rates of *T. urticae* females treated with spirodiclofen (mg/L) in pre-ovipositional period
Slika 1. Stope preživljavanja ženki *T. urticae* tretiranih spirodikofenom kao adulti starosti 2.5-3.5 dana

Total fecundity/fertility of *T. urticae* significantly decreased as concentrations of spirodiclofen increased (Table 2). The highest concentration, 96 mg/L, completely terminated egg-laying, while among the

Table 1. Survival and fecundity of *T. urticae* females exposed to spirodiclofen (mg/L) for 24 h
Tabela 1. Preživljavanje i fekunditet ženki *T. urticae* izloženih 24 h spirodiklofenu (mg/L)

mg/L	*FT*	*FS*	P_{FS}	*FS'*	*E*	*E'*
0	25	25	1.00	25	218	8.72 *a*
6	35	30	0.86	32.5	308	9.48 *a*
12	45	32	0.71	38.5	196	5.09 *b*
24	55	30	0.54	42.5	170	4.00 *b*
48	70	35	0.50	52.5	126	2.40 *c*
96	85	37	0.44	61	114	1.87 *c*

FT = Number of treated females/Broj tretiranih ženki
FS = Number of females that survived 24 h exposure/Broj ženki preživelih 24-časovnu ekspoziciju
P_{FS} = Proportion of females that survived treatment; $P_{FS} = FS/FT$/Proporcija ženki koje su preživele tretman
FS' = Number of living females at mid-interval of exposure; $FS' = ½ (FT + FS)$/Broj živih ženki na sredini intervala ekspozicije
E = Total number of eggs laid during exposure/Ukupan broj jaja položen za vreme ekspozicije
E' = Mean number of eggs laid per female at mid-interval; $E' = E/FS'$/Prosečan broj jaja položen po ženki na sredini intervala
Data transformed by $\sqrt{n}$, means separated by *t*-test, $p<0.05$/Transformacija podataka $\sqrt{n}$, *t*-test, $p<0.05$

Table 2. Total fecundity (T_L) and fertility (T_H) of *T. urticae* females surviving treatment with spirodiclofen (mg/L) as 2.5-3.5 days old adults
Table 2. Ukupan fekunditet (T_L) i fertilitet (T_H) ženki *T. urticae* preživelih tretiranje spirodiklofenom (mg/L) kao adulti starosti 2.5-3.5 dana

mg/L	T_L	N_L	T_H	N_H
0	51.17 *a*	25	46.92 *a*	25
6	41.72 *a*	25	25.72 *b*	21
12	30.28 *b*	16	19.44 *b*	15
24	4.52 *c*	3	2.00 *c*	3
48	3.72 *c*	3	1.92 *c*	2
96	0.00 *d*	0	0.00 *c*	0

Fecundity/fertility data transformed by √(n + 0.5); means separated by *t*-test, p<0.05
Transformacija podataka o fekunditetu/fertilitetu √(n+0.5), *t*-test, p<0.05

N_L = Number of females that laid eggs in a cohort of 25 females/Broj ženki koje su polagale jaja u kohorti od 25 ženki
N_H = Number of females that laid viable eggs in a cohort of 25 females/Broj ženki koje su polagale vitalna jaja u kohorti od 25 ženki

females that survived 48 mg/L and 24 mg/L only two and three females, respectively, laid viable eggs. On the other hand, 60% and 84% of the female survivors of treatments with 12 mg/L and 6 mg/L concentrations, respectively, laid viable eggs; total fertility of these females was respectively reduced by 58.6% and 45.2%. All untreated females laid viable eggs. In our previous work with females treated in the pre-ovipositional stage (Marčić, 2007), the highest concentration was also found to terminate egg-laying.

On the first day after treatment (Table 3), females treated with 24 mg/L, 12 mg/L and 6 mg/L laid eggs; viable eggs were laid only by the latter and the percentage of hatching was merely 3.1% (89% in control). On the second day, females treated with 48 mg/L also began to lay eggs, but viable eggs were laid only by females treated with 12 mg/L and 6 mg/L (the percentage of hatching was 28.5% and 65.3%, respectively; 93.9% in control). From the third day onward, viable eggs were laid also by females treated with 48 mg/L, and the difference in hatchability was considerably smaller or disappeared completely. Compared to control, gross fecundity was reduced by all concentrations significantly only on the first day of experiment, and gross fertility in the first two days. By the end of the experiment, there was no significant difference between gross fecundity/fertility of untreated females and those treated with 6 mg/L and 12 mg/L concentrations. However, all concentrations significantly reduced net fecundity/fertility throughout the trial (Table 4), which indicates a considerable impact of the decreased female survival rate on overall net fer-

Table 3. Gross fecundity (G_L) and gross fertility (G_H) of *T. urticae* females surviving treatment with spirodiclofen (mg/L) as 2.5-3.5 days old adults
Tabela 3. Bruto fekunditet (G_L) i bruto fertilitet (G_H) ženki *T. urticae* preživelih tretiranje spirodiklofenom (mg/L) kao adulti starosti 2.5-3.5 dana

x	3.5-4.5		4.5-5.5		5.5-6.5		6.5-7.5		7.5-8.5	
mg/L	G_L	G_H	G_L	G_H	G_L	G_H	G_L	G_H	G_L	G_H
0	12.88 *a*	11.46 *a*	14.29 *a*	13.42 *a*	9.95 *b*	8.59 *a*	7.82 *ab*	7.50 *ab*	10.28 *a*	9.72 *a*
6	9.00 *b*	0.28 *b*	9.56 *b*	6.24 *b*	11.15 *ab*	9.05 *a*	10.00 *a*	8.25 *a*	8.21 *ab*	7.05 *ab*
12	2.24 *c*	0.00 *b*	10.33 *ab*	2.94 *c*	12.62 *a*	9.88 *a*	9.56 *a*	8.75 *a*	10.00 *a*	9.00 *a*
24	0.20 *d*	0.00 *b*	2.31 *c*	0.00 *d*	3.40 *c*	1.40 *b*	2.56 *bc*	1.89 *bc*	2.62 *bc*	2.38 *bc*
48	0.00 *d*	0.00 *b*	1.33 *c*	0.00 *d*	3.00 *c*	1.00 *b*	2.67 *bc*	2.11 *bc*	3.71 *bc*	2.86 *bc*
96	0.00 *d*	0.00 *b*	0.00 *c*	0.00 *d*	0.00 *d*	0.00 *b*	0.00 *c*	0.00 *c*	0.00 *c*	0.00 *c*

Fecundity/fertility data transformed by √(n + 0.5); means separated by *t*-test, p<0.05
Transformacija podataka o fekunditetu/fertilitetu √(n+0.5), *t*-test, p<0.05

x = Female adult age (days)/Starost odraslih ženki (dani)

Table 4. Net fecundity (N_L) and net fertility (N_H) of *T. urticae* females surviving treatment with spirodiclofen (mg/L) as 2.5-3.5 days old adults
Tabela 4. Neto fekunditet (N_L) i neto fertilitet (N_H) ženki *T. urticae* preživelih tretiranje spirodiklofenom (mg/L) kao adulti starosti 2.5-3.5 dana

x	3.5-4.5		4.5-5.5		5.5-6.5		6.5-7.5		7.5-8.5	
mg/L	N_L	N_H	N_L	N_H	N_L	N_H	N_L	N_H	N_L	N_H
0	12.88 *a*	11.46 *a*	14.29 *a*	13.42 *a*	9.15 *a*	7.90 *a*	7.19 *a*	6.90 *a*	7.71 *a*	7.29 *a*
6	7.74 *b*	0.24 *b*	8.22 *b*	5.37 *b*	7.69 *b*	6.24 *b*	6.90 *a*	5.69 *a*	5.34 *b*	4.58 *b*
12	1.59 *c*	0.00 *b*	5.27 *c*	1.50 *c*	5.68 *c*	4.45 *c*	4.30 *b*	3.94 *b*	4.30 *c*	3.87 *c*
24	0.11 *d*	0.00 *b*	0.67 *d*	0.00 *d*	0.75 *d*	0.31 *d*	0.51 *c*	0.38 *c*	0.47 *d*	0.43 *d*
48	0.00 *d*	0.00 *b*	0.32 *d*	0.00 *d*	0.54 *d*	0.18 *d*	0.48 *c*	0.38 *c*	0.52 *d*	0.40 *d*
96	0.00 *d*	0.00 *b*	0.00 *d*	0.00 *d*	0.00 *d*	0.00 *d*	0.00 *c*	0.00 *c*	0.00 *d*	0.00 *d*

Fecundity/fertility data transformed by $\sqrt{(n + 0.5)}$; means separated by *t*-test, $p<0.05$
Transformacija podataka o fekunditetu/fertilitetu $\sqrt{(n+0.5)}$, *t*-test, $p<0.05$

x = Female adult age (days)/Starost odraslih ženki (dani)

tility reduction, especially from the third day onward. Spirodiclofen was found to have similar effect on the fecundity and fertility of *T. urticae* pre-ovipositional females (Marčić, 2007).

Spirodiclofen considerably affected the viability, fecundity and fertility of two-spotted spider mite females that survived treatment. Reproduction of the females treated with 96 mg/L was terminated after 24 h of exposure and most females died within five days. Total fertility of the females treated with 48 mg/L and 24 mg/L concentrations was reduced by 95.9% and 95.7%, respectively. Spirodiclofen effects were most evident on the first day after treatment, when only females treated with the lowest concentration laid viable eggs. Reduced fecundity of *T. urticae* females that survived exposure to spirodiclofen was also reported by Wachendorff et al. (2002) and Cheon et al. (2007). Wachendorff et al. (2002) reported a reduction in fecundity to almost zero after short-term (1-2 h) tarsal exposure of females to 440 ng a.i./cm^2 of spirodiclofen. On the other hand, Cheon et al. (2007) found that the number of eggs laid by females exposed for seven days to 180 mg/L of the acaricide was reduced by 87%; the authors, however, did not specify the amount of spirodiclofen deposit. In our bioassay, no eggs were laid after 24 h exposure to 96 mg/L (i.e. 408 ng a.i./cm^2, assuming aqueous deposit of 4.25 mg/cm^2), while 48 mg/L (204 ng a.i./cm^2) and 24 mg/L (102 ng a.i./cm^2) caused fertility to drop to below 5%. Previously we reported (Marčić and Ogurlić, 2006b) that *T. urticae* females exposed for 2 h to 240 ng a.i./cm^2 of spirodiclofen had fertility reduced by 27%; an exposure extended to 6 h and 24 h reduced net fertilitet by 63% and 91%, respectively.

In several European countries, spirodiclofen has been found to provide good efficacy against two-spotted spider mite when applied at 96 mg/L concentration (Elbert et al., 2002). On the other hand, laboratory studies and field trials with concentrations 48-96 mg/L and/or rates up to 240 g/ha (Wolf and Schnorbach, 2002; De Maeyer et al., 2002; Hardman et al., 2003; Reis et al., 2006; Welty, 2006) revealed some adverse effects on the survival and fecundity of several predatory mite species. In this study, treatment with 96 mg/L terminated reproduction of *T. urticae*, while 48 mg/L, 24 mg/L and 12 mg/L concentrations of spirodiclofen reduced total fertility to 4.1%, 4.3% and 41.4%, respectively. Treatment with 6 mg/L, i.e. 102 ng a.i./cm^2 (the concentration/dose discriminative for eggs and immatures, i.e. around 90% of population) nearly halved total fertility and significantly reduced net fertility. Spirodiclofen also achieved similar effect after treatment of two-spotted spider mite pre-ovipositional females with the same series of concentrations/doses (Marčić, 2007), indicating that female age does not crucially affect the effectiveness of this acaricide.

Rates below those recommended may be used in combination with biological control agents within an integrated pest management (IPM) system (Roush, 1989; Wege and Leonard, 1994; Hoy, 1995; Dent, 2000), reducing the selection pressure and development of resistance. Rhodes et al. (2006) showed that releasing of phytoseiid mites after applying bifenazate at half the recommended rate effectively controlled *T. urticae* in strawberries. The findings field studies with spirodiclofen applied at recommended rates (Wolf and Schnorbach, 2002; De Maeyer et al., 2002) nevertheless led the

authors to conclude that the observed adverse effects on predacious mites do not compromise the IPM compatibility of this acaricide. However, looking from the aspect of stable age distribution of two-spotted spider mite, spirodiclofen applied at lower rates (i.e. at concentration discriminative for eggs and immatures) could be more successfully combined with phytoseiid mites release and thus improve the management of *T. urticae* populations.

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Efekti spirodiklofena na reprodukciju običnog paučinara (*Tetranychus urticae* Koch)

REZIME

Efekti spirodiklofena na preživljavanje, fekunditet i fertilitet ženki običnog paučinara (*Tetranychus urticae* Koch), preživelih tretiranje u uzrastu adulta starih tri dana serijom koncentracija počevši od koncentracije diskriminativne za jaja i nezrele stadijume, ispitivani su u laboratorijskom ogledu. Nakon ekspozicije od 24 časa, proporcija ženki koje su preživele tretman bila je 0.86 (6 mg/L), 0.71 (12 mg/L), 0.54 (24 mg/L), 0.50 (48 mg/L) i 0.44 (96 mg/L). U narednih pet dana, stope preživljavanja ženki tretiranih koncentracijom 6 mg/L, odnosno 12 mg/L, bile su znatno ispod stopa preživljavanja netretiranih ženki, ali su se zadržale iznad stopa preživljavanja ženki tretiranih ostalim koncentracijama. Ukupni fekunditet/fertilitet opadao je značajno, kako se koncentracija akaricida povećavala. Najviša koncentracija, 96 mg/L, potpuno je zaustavila polaganje jaja, dok su samo dve, odnosno tri ženke, među onima koje su preživele tretiranje koncentracijom 48 mg/L, odnosno 24 mg/L, polagale vitalna jaja. S druge strane, 60%, odnosno 84% ženki preživelih tretiranje koncentracijom 12 mg/L, odnosno 6 mg/L polagalo je vitalna jaja; ukupan fertilitet tih ženki bio je redukovan za 58.6%, odnosno 45.2%. Prvog dana nakon tretmana, ženke tretirane koncentracijom 24 mg/L, 12 mg/L, odnosno 6 mg/L, polagale su jaja; vitalna jaja polagale su samo ove poslednje, a procenat piljenja bio je svega 3.1% (89% u kontroli). Drugog dana, i ženke tretirane koncentracijom 48 mg/L počele su da polažu jaja, ali su vitalna jaja polagale samo ženke tretirane koncentracijom 12 mg/L, odnosno 6 mg/L (odgovarajući procenti piljenja iznosili su 28.5%, odnosno 65.3%; 93.9% u kontroli). Od trećeg dana pa nadalje, i ženke tretirane koncentracijom 48 mg/L polagale su vitalna jaja, a razlika u procentima piljenja između tretiranih i netretiranih ženki bila je znatno manja ili je potpuno nestala. U poređenju sa kontrolom, sve koncentracije su značajno redukovale bruto fekunditet samo prvog, a bruto fertilitet samo u prva dva dana nakon tretmana. Do kraja ogleda nije registrovana statistički značajna razlika u bruto fekunditetu/fertilitetu između netretiranih i ženki tretiranih koncentracijom 6 mg/L, odnosno 12 mg/L. Međutim, sve koncentracije su ostvarile značajnu redukciju neto fekunditeta/fertiliteta u svih pet dana trajanja ogleda, što ukazuje na znatan doprinos sniženih stopa preživljavanja ukupnoj redukciji neto fertiliteta, posebno počev od trećeg dana pa do kraja ogleda. Subletalni efekti spirodiklofena razmatrani su u kontekstu suzbijanja populacija *T. urticae*.

Ključne reči: *Tetranychus urticae*, spirodiklofen, reprodukcija