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COMMUNICATIONS IN BIOMETRY AND CROP SCIENCE
Vol. 11, NO. 2, 2016, PP. 140–148

INTERNATIONAL JOURNAL OF THE FACULTY OF AGRICULTURE AND BIOLOGY,
Warsaw University of Life Sciences – SGGW, POLAND

REGULAR ARTICLE

Side effects of acequinocyl on predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)

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CITATION: Puchalska E., Piotrowska M. (2016). Side effects of acequinocyl on predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). *Communications in Biometry and Crop Science* 11, 140–148.

Received: 4 April 2016, Accepted: 22 July 2016, Published online: 3 August 2016

ABSTRACT

Contact and residual effects of acequinocyl on life parametres of *Typhlodromus pyri* females were studied under laboratory conditions. The pesticide toxicity varied depending on the mode of exposure and *T. pyri* population origin. Acequinocyl proved to be highly toxic to *T. pyri* females from U-population that had never been in contact with any pesticide. 100% mortality of these females was observed on the third day after spraying leaves alone (residual effect) and on the fifth day after females' treatment (direct-contact effect). Another tested population of *T. pyri* (S-population) was established with individuals collected in commercial apple orchard where various pesticides were often applied. Using Baillod and Lenfant formula to estimate global effect of acequinocyl on S-population of *T. pyri*, the acaricide was established as slightly toxic for exposure to leaf surface residues, with GE-value 26.86, and moderately toxic when exposed by direct contact, with GE-value 46.67.

Key Words: *predatory mite; toxicity; side effects; acaricides.*

INTRODUCTION

Phytoseiids, especially *Typhlodromus pyri* Scheuten, are the most frequently used biocontrol agents against herbivorous mites occurring in orchards of central Europe (Duso 1992, Sengonca et al. 1994, Blommers 1994, Solomon et al. 2000, Praslicka et al. 2011). Wide range of preys, including spider mites, eriophyoids, tydeids or tenuipalpids (Zemek 1993, Vargas and Cardemil 2005, Lorenzon et al. 2012), as well as ability to develop on supplementary food, i.e. pollen (Duso and Camporese 1991), make *T. pyri* capable of building up stable populations that are less dependent on dispersal to new sites in search of prey than specialists (McMurtry 1992). However, the predator's population stability, thus its

effectiveness, is also affected by pesticides used in integrated pest management conducted in orchards (Kreiter et al. 2010). *Typhlodromus pyri* is known to build up resistance to pesticides, even to those compounds that cause high mortality rates such as pyrethroids or organophosphorous insecticides (Overmeer and Van Zon 1983, Vidal and Kreiter 1995, Bonafos et al. 2007). On the other hand, there are several examples of pesticide side effects on *T. pyri*, including increased mortality of different stages and reduced fertility of females (Overmeer and Zon 1981, Blümel et al. 2000, Kreiter et al. 2010).

The application of plant protection products of low toxicity to natural enemies and the use of pesticide-resistant predatory mites are a prerequisite for successful integration of chemical and biological methods (Markwick 1986, Hassan et al. 1991). Therefore, knowledge of the impact of individual pesticides on beneficial organisms is important to their utility in IPM programs (Sandez-De-Cabezon Irigaray and Zalom 2006, Salman et al. 2015).

Acequinocyl (3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate) is presently the only commercial acaridae of the naphtoquinone analogue group, designated as a "reduced risk" pesticide by the United States Environmental Protection Agency (U.S. EPA). Acequinocyl inhibits mitochondrial respiration (METI group) and has been introduced for the control of many phytophagous mites (Salman et al. 2015). Toxicity of this pesticide to phytoseiides, such as *Phytoseius persimilis* A.H., *Galenodromus occidentalis* (Nesbitt) or *Amblyseius womersleyi* Schicha, has been already studied (Kim and Seo 2001, Sandez-De-Cabezon Irigaray and Zalom 2007), but its influence on one of the most important naturally occurring in Europe predatory mite, *T. pyri*, is unknown. Therefore, in this study we have assessed the contact and residue effect of acequinocyl on *T. pyri* survival and reproduction.

MATERIALS AND METHODS

COLLECTION AND REARING OF MITES POPULATIONS

The population of two-spotted spider mite, *Tetranychus urticae* Koch, was obtained from collections made in 2001 on *Phaseolus vulgaris* L., at the Department of Applied Entomology, SGGW, Warsaw. The rearing was conducted in laboratory conditions. Seedlings were planted in plastic containers (40 x 25 x 9 cm) and infested with all development stages of *T. urticae*. Every 6 days, new bean plants were added to maintain the population.

Two separate stock colonies of *T. pyri* was maintained in the environmental test chambers (Sanyo MLR-350; 23 ± 2°C, 75 ± 10% RH and 16L:8D photoperiod). First of them (S-population) was initiated with specimens obtained from commercial apple orchard in Skierniewice (Poland) sprayed in previous years with acaricides, i.e. Ortus® 05 SC (fenpyroximate), Sanmite® 20 WP (pyridaben), Sumo® 10 EC (milbemectin) and Envidor® 240 SC (spirodiclofen). The second mass culture of *T. pyri* (U-population) was established with individuals collected from *Tilia cordata* trees growing in Ursynów Park in Warsaw (Poland). These trees have never been treated with any pesticides.

Both populations were reared separately on detached apple leaves (cv. 'Golden Delicious Reinders') placed upside-down on plastic plates (12x17cm) resting on a wet sponge in open plastic trays (21 x 15 x 9 cm). To keep the sponge wet and to prevent the mites from escaping, water was added to the boxes. Additionally strips of wet tissue paper were placed on leaves to provide water. Typha pollen (*Typha latifolia* L.) and all stages of *T. urticae* obtained from the culture described above were offered to predators as food. Phytoseiids were reared for at least five generations before conducting the experiment.

EXPERIMENTAL UNIT

The experimental unit was a modified Munger cell (Overmeer 1985). The cell consisted of a stack of four 100 x 50 mm layers, in the following order: 2 mm thick bottom Plexiglas plate covered with tissue paper, a detached apple leaf placed upside-down on the tissue paper, 7 mm thick plate with a 30 mm hole in the center sealed with plasticine, and 2 mm

thick top plate with a 10 mm ventilation hole, covered with muslin mesh. The plasticine was used to prevent mites from escaping from the arena. The stack was held together with rubber bands. To maintain humidity in the cell, the tissue paper was moistened daily with distilled water. A piece of a transparent plastic sheet folded in the shape of a tent was placed over each arena as a shelter and oviposition site for phytoseiids.

TOXICOLOGICAL TESTS

To obtain the required quantity of females for testing, 160 deutonymphs of *T. pyri* (80 individuals from each stock colony) were maintained in Petri dishes fed eggs of *T. urticae*. After 2-3 days, males were introduced to these dishes for 12 hours to provide mating opportunities to the newly emerged females.

The tested acaricide was Kanemite® 150 SC (acequinocyl 14.42%) used at the maximum dosage suggested for field application (1.87 l ha^{-1}; 280.5g ai ha^{-1}). The product mixed with distilled water was applied using a 200-ml hand sprayer held 30 cm away from the leaf surface (Sandez-De-Cabezon Irigaray and Zalom 2006). The untreated controls were sprayed with distilled water alone.

Residual and direct-contact effect of pesticide was assessed using 90 females of each population (10 females per treatment in three replicates per combination). In residual tests, leaves alone were sprayed as described above. After the pesticide solution had dried up, *T. pyri* females were placed individually on treated leaves. In direct-contact tests, females were placed on detached apple leaves before spraying.

After treatment, leaves with females were placed in experimental units and kept in the test chamber (Sanyo MLR-350; 23 ±2°C, 75 ±10% RH and 16L:8D photoperiod). Every 24 hours, fifteen *T. urticae* eggs were added to each experimental unit to provide food for the predator.

Egg laying and female mortality was checked every 24 hours during 10 days. Protonymphs (F1) were removed from experimental units and their viability was evaluated. Mites were considered dead if they were unable to react when gently prodded with a fine brush. At the end of the experiment, the global effect (GE), ranging from 0 to 100%, was calculated according to the Baillod and Lenfant (1994) formula: $GE = 100 - (100 - M) \times R1 \times R2$, where M is mortality (%) corrected for control mortality by Abbott's (1925) formula, R1 is the corrected fecundity index, and R2 the corrected offspring survival index. R1, the corrected fecundity index, equals $RT \times RC^{-1}$, where RT is the fecundity in the treated group and RC is the fecundity in the control group. R2, the corrected offspring survival index, equals $OSIT \times OSIC^{-1}$, where the offspring survival index (OSI) in treated (OSIT) and control (OSIC) groups is expressed by the numbers of nymphs removed between days 0 and 10, divided by the numbers of eggs + larvae (on day 10) plus the numbers of nymphs removed in this period (Kreiter et al. 2010). The GE scale following Kreiter et al. (2010) is:

0<GE<19%, neutral pesticide (N)
20<GE<39%, slightly toxic pesticide (ST)
40<GE<59%, moderately toxic pesticide (MT)
60<GE<79%, toxic pesticide (T)
GE>80%, highly toxic pesticide (HT).

DATA ANALYSIS

The statistical evaluation was carried out with the statistical software package PAST version 2.02. (Copyright© Kammer & Harper 1999-2010). Differences in female survival in each tested combination were studied with the help of a generalized linear model with binomial distribution and logit link function. Reproductive parameters were tested with F test of ANOVA, and means were compared using the Student-Newman-Keuls test. Toxicity factors: M, R1 and R2 were compared using Mann-Whitney (Wilcoxon) W test. The significance level for all analyses was 0.05.

RESULTS AND DISCUSSION

Typhlodromus pyri is a predator commonly used against spider mites and rust mites occurring on apples (Van de Vrie 1985, Maixner 1990). Therefore, testing its sensitivity to pesticides used in apple orchards is greatly needed. The present studies demonstrate direct and residual effect of one of such pesticides, acequinocyl, on mortality and fecundity of *T. pyri* females and survival of their progeny. The pesticide toxicity varied depending on the mode of exposure and *T. pyri* population origin.

Acequinocyl proved to be highly toxic to *T. pyri* females from U-population (originated from linden trees growing in urban park). Surviving of these females recorded 24 hours after spraying was significantly lower in both pesticide-treated groups (residual and direct-contact effect) than in control group (70%, 20% and 100% respectively; P<0.001; Fig. 1A). On the third day after spraying, none of females remained alive in the directly treated group (Fig. 1B). Also in samples testing residual effect of pesticide, mortality of tested females increased over time up to 100% after 5 days of experiment. Because no females survived, direct and residual treatment, and fecundity and progeny survival could not be determined.

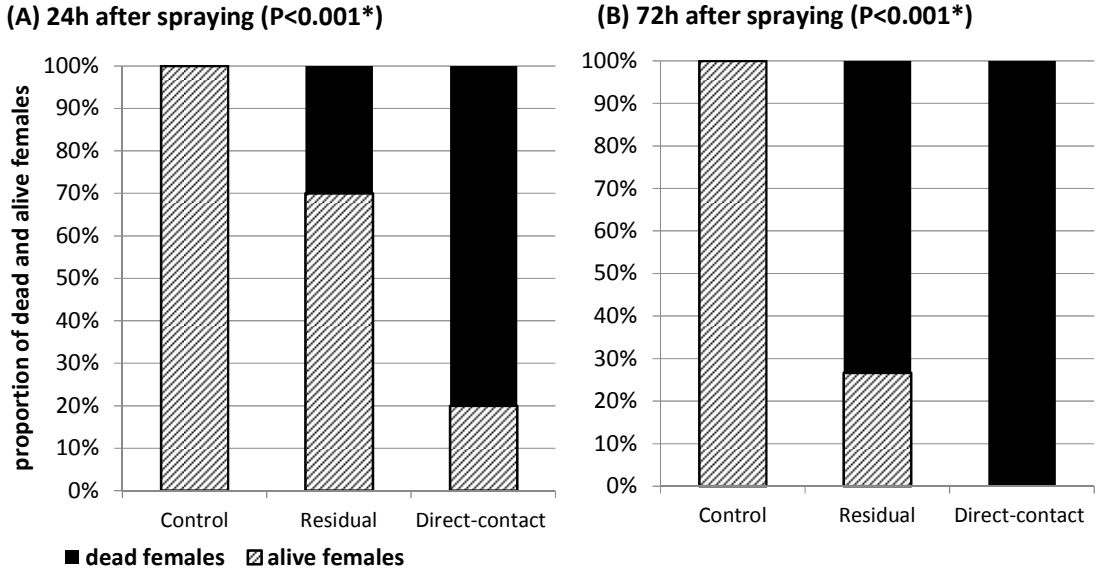

Figure 1. The effect of acequinocyl on survival of *T. pyri* females from U-population 24 hours after spraying (A) and 73 hours after spraying (B) (*generalized linear models).

High susceptibility of *T. pyri* U-population to acequinocyl could result from the fact that these predators have never been in contact with any pesticides. Our results are consistent with findings of Bonafos et al. (2007), who reported that the same dose of deltamethrin caused 100% mortality of *T. pyri* population from pesticide-free orchard, and only 30% mortality of population coming from the orchard where insecticides were often applied. Similarly Sandez-De-Cabezon Irigaray et al. (2007) using phytoseiid colonies never treated with acaricides revealed that exposure to acequinocyl residues 3 days after leaves' treatment resulted in 100% mortality of *P. persimilis* and *G. occidentalis*. Female fecundity was strongly decreased even following exposure to leaves treated with acequinocyl 6 days earlier.

In the case of *T. pyri* S-population (established with individuals collected in commercial apple orchard where METI acaricides were often applied), assessment of female survival 5 days following pesticide application did not show significant differences between control and residual or direct exposure to acequinocyl (P=0.066; Fig. 2A). Ten days after exposure to

the pesticide, in control group 88% of tested females were alive while 84% and 75% of tested females survived in treated samples (in residual and direct-contact tests, respectively) (Fig. 2B).

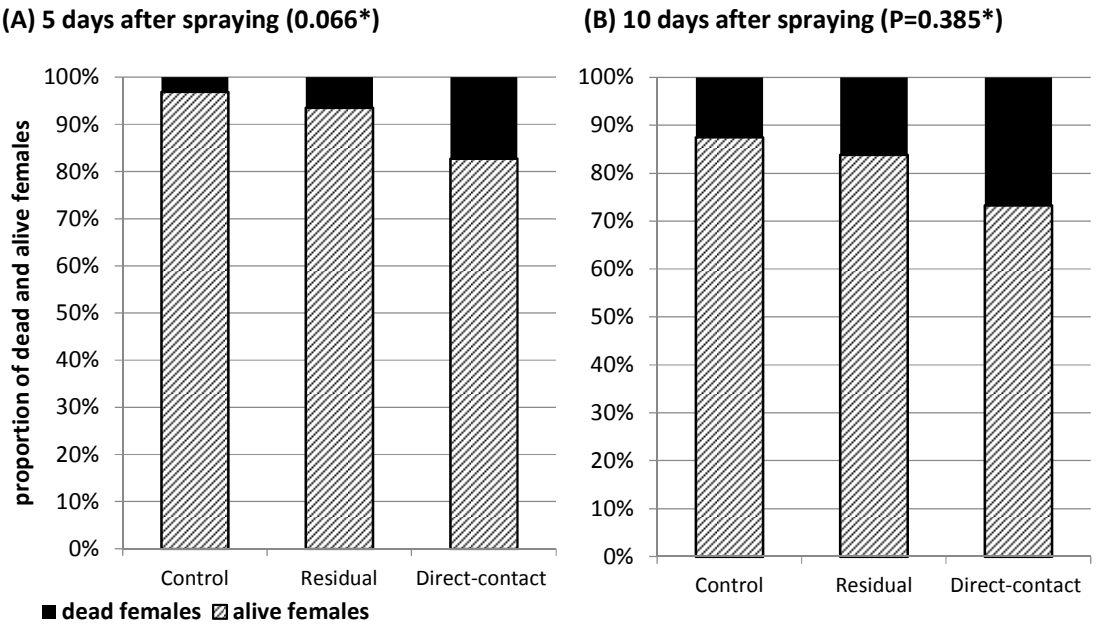

Figure 2. The effect of acequinocyl on survival of *T. pyri* females from S-population five days after spraying (A) and ten days after spraying (B) (*generalized linear models).

Still, the differences observed between combinations were not statistically significant (P=0.385). Kim and Yoo (2002), who assessed side effect of acequinocyl on another predatory mite *P. persimilis*, also reported high females' survival ranging 80-94%, even in directly treated samples. Moreover, these authors observed that *P. persimilis* females treated with acequinocyl produced 88% as many eggs as did control females (Kim and Yoo 2002). Our studies indicate that reproductive potential of surviving *T. pyri* females of S-population was significantly affected by acequinocyl. Residual and direct-contact effect of tested pesticide on reproductive parameters of *T. pyri* females from S-population are shown in Table 1. Untreated females had preoviposition period of 0.6 days and reached the oviposition peak 48 hours after spraying with distilled water. Preoviposition period of females treated with acequinocyl (both modes of exposure) was about 5 times longer than in control group ($F=42$; $P<0.001$). The oviposition peak occurred in pesticide treated samples 96 hours after spraying. Moreover acequinocyl had deleterious impact on female fecundity ($F=15.85$; $P=0.004$; Table 1). Exposure to acequinocyl residues reduced female fecundity by 40%. The effect of pesticide was higher during direct exposition, when females produced only 52% as many eggs as did control females. No significant differences among treatments were observed regarding egg-laing rates (number of eggs /female /day) ($F=1.88$; $P=0.180$; Table 1). These results are consistent with the findings of Sandez-De-Cabezon Irigaray and Zalom (2006), who reported significant influence of acequinocyl on fecundity of *M. occidentalis* females. However, further surveys testing the effect of acequinocyl on this phytoseiid mite had shown its increasing resistance to the pesticide (Zalom and Sandez-De-Cabezon Irigaray 2010).

Based on Baillod and Lenfant (1994) formula to estimate global effect of testing pesticide on S-population of *T. pyri*, acequinocyl can be considered slightly toxic for exposure to leaf surface residues, with GE-value of 26.86, and moderately toxic when exposed by direct contact, with GE-value of 46.67 (Table 2). Higher values of corrected mortality (M) and lower

corrected fecundity (R1) of surveyed females were observed during direct contact with pesticide than when phytoseiids were exposed to leaf surface residues (P=0.006 and P=0.008 for M and R1, respectively). The corrected offspring survival index (R2) did o't differ in both testing combinations (P=0.239; Table 2). Preliminary observations concerning the influence of acequinocyl on *T. pyri* abundance in commercial orchards of Nova Scotia (Canada) also indicated slight toxicity of the acaricide to the predator (Hardman and Bostanian 2010).

Table 1. Residual and direct-contact effect of acequinocyl on reproductive parameters of *T. pyri* females from S-population (F test of ANOVA; a,b- groups obtained with Student-Newman-Keuls test for P<0.05).

	Fecundity			Oviposition rate			Preoviposition period		
	number of eggs / female / 10 days	±SD		number of eggs / female / day	±SD		number of days before laying first egg	±SD	
Control	4.6	0.54	a	0.65	0.22	a	0.6	0.56	a
Residual effect	2.8	0.44	b	0.40	0.35	a	2.8	0.45	b
Direct-contact effect	2.4	0.89	b	0.34	0.38	a	3.2	0.45	b
F	15.85			1.88			42		
P-value	0.0004			0.1807			<0.001		

Table 2. Global effect of acequinocyl on S-population of *T. pyri*. (toxicity factors: R1- corrected fecundity index; R2 - corrected offspring survival index; a,b- groups obtained with Mann-Whitney (Wilcoxon) W test for P<0.05).

	Corrected Mortality (%)	R1	R2	Global effect (GE) (%)	Toxicity Class	
					Symbol	name
Residual effect	4.1 a	0.82 b	0.93 a	26.86	ST	slightly toxic
Direct-contact effect	17.2 b	0.7 a	0.92 a	46.67	MT	moderately toxic
Test statistic	30	0	3.5			
P-value	0.006	0.008	0.239			

S-poulation of *T. pyri* originated from the orchard where some METI acaricides i.e. fenpyroximate and pyridaben were applied. Nevertheless, both these substances are mitochondrial electron transport inhibitors at Complex I (Lummen 2007) whereas acequinocyl is an inhibitor of Q_0 center by acting as a structural analogue of ubiquinone at Complex III (Kinoshita et al. 1999). Also other pesticides used in this orchard like milbemectin and spirodiclofen represent modes of action different to acequinocyl (GLUCL allosteric modulators and inhibitors of acetyl CoA carboxylase, respectively). However, quite low susceptibility of *T. pyri* S-population to acequinocyl could be the result of cross-resistance to some of these pesticides. Cross-resistance to acequinocyl and milbemectin was previously documented by Salman et al. (2015) in *P. persimilis* population from Turkey.

CONCLUSIONS

Integrating of biological and chemical controls is essential to implementation of Integrated Mite Management programs in crops where pesticide intervention is routinely used by farmers for control of key pest species (Zalom and Sandez-De-Cabezon Irigaray 2010). Our study demonstrates that acequinocyl is slightly or moderately toxic to *T. pyri* individuals that previously were in contact with pesticides. We can expect that the side effect of acequinocyl on the predator would be lower in field conditions. Results from laboratory tests are usually overestimated because of high exposure of females to pesticides, whereas in orchards predators have various shelters and can be more dispersed to untreated plant surfaces (Bonafos et al. 2007). Thus, the results of our laboratory tests suggest that acequinocyl is promising candidate for use in integrated mite management programs where *T. pyri* is the major natural enemy.

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