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Field and greenhouse evaluation of spirodiclofen against *Panonychus ulmi* and *Tetranychus urticae* (Acari: Tetranychidae) in Serbia*

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Abstract

Spirodiclofen, an acaricide with a novel mode of action (inhibition of lipid synthesis), has recently been commercialized and recommended as a compound that effectively controls mite populations resistant to other acaricides. The efficacy of the spirodiclofen against European red mite [*Panonychus ulmi* (Koch)] on apple, and two-spotted spider mite (*Tetranychus urticae* Koch) on greenhouse cucumber was tested in Serbia from 2004 to 2008. Spirodiclofen efficacy was compared to the effectiveness of several other acaricides (bifenthrin, clofentezine, fenazaquin, fenpyroximate) that had been in use for some time. The acaricides were applied at their recommended application rates. Control efficacy against *P. ulmi* was tested in a commercial apple orchard at Morovic (45°00.711'N; 19°15.146' E). Applied at the BBCH 09 growth stage (green leaf tips about 5 mm above bud scales) against overwintering eggs, spirodiclofen achieved 89.1 and 86.0% efficacy in evaluations 12 and 25 DAT (days after treatment), respectively. Similar effectiveness was demonstrated for fenazaquin (91.3 and 84.4%), while clofentezine achieved a considerably lower efficacy (67.4 and 27.8%, respectively). In three trials involving the summer population of *P. ulmi* in three vegetation seasons, spirodiclofen achieved high and steady efficacy: 91% (15 DAT), 97.2% (30 DAT) and 98% (45 DAT) in the first season; 95.2% (14 DAT), 96.3% (29 DAT) and 95.7% (47 DAT) in the second season; and 97.4% (14 DAT), 95.6% (21 DAT) and 97% (38 DAT) in the third season. The highest clofentezine efficacy in the first season was 90.9% (30 DAT), 77.4% (29 DAT) in the second and 68.1% (21 DAT) in the third season. Bifenthrin achieved the highest efficacy in the first season, 78.2% (15 DAT), while it declined to 65.9% (14 DAT) in the second and was practically negligible in the third season. Such unsatisfactory efficacies of bifenthrin and clofentezine were probably the result of resistance development under selection pressure of these compounds at Morovic. The efficacy of fenpyroximate, tested in the second season, was high (97.4%) in evaluations 14 and 29 DAT, but it was only 50.3% in evaluation 47 DAT. Efficacy in controlling *T. urticae* was tested in a commercial greenhouse in Padinska Skela (44°57.012' N; 20°25.741' E). In evaluations 6 and 10 DAT, spirodiclofen achieved 98.4 and 96.8% efficacy, while clofentezine effectiveness was 95.4 and 93.4%, and bifenthrin efficacy 96.5 and 98.8%, respectively. The results showed that spirodiclofen is effective in controlling European red mite on apple and two-spotted spider mite on cucumber, and a good alternative to older acaricides.

Key words: spirodiclofen, *Panonychus ulmi*, *Tetranychus urticae*, chemical control.

Introduction

Spider mite (Acari: Tetranychidae) resistance to acaricides has become a global phenomenon as a result of their exceptional intrinsic potential to evolve resistance and human activities contributing to it. A way of dealing with the resistance is to search for new compounds acting on novel biochemical and physiological targets (Dekeyser, 2005; Whalon *et al*., 2008; van Leeuwen *et al*., 2009). Spirodiclofen, a tetronic acid derivative, is the first member of a class of acaricides/insecticides with new mode of action—inhibition of lipid biosynthesis. This compound exhibits activity against eggs and immatures of spider mites, while its activity against adult females is slower, with strong reduction of fecundity and fertility (Wachendorff *et al*., 2002;

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Bretschneider *et al*., 2007; Marcic, 2007; van Pottelberge *et al*., 2009). Spirodiclofen has been commercialized for worldwide use against all important phytophagous mites in perennial and row crops (Elbert *et al*., 2002; Bretschneider *et al*., 2007). Owing to its new mode of action, spirodiclofen is characterized by lack of cross-resistance to any other commercial acaricide, and is recommended as an effective option for controlling resistant mite populations (Dekeyser, 2005; Bretschneider *et al*., 2007).

This paper presents the results of tests on the efficacy of spirodiclofen against European red mite [*Panonychus ulmi* (Koch)] on apple and two-spotted spider mite (*Tetranychus urticae* Koch) on greenhouse cucumber, carried out in Serbia. The efficacy of spirodiclofen was compared to other acaricides already in use and the results are discussed with a view to improving the control of *P. ulmi* and *T. urticae* populations.

Materials and Methods

Biological efficacy of spirodiclofen (commercial product Envidor®, 240 g/L a.i., Bayer CropScience) and five other acaricides used for spider mite control was tested in four field trials (F1–F4) and one greenhouse trial (GH) (Tables 1, 2). The trials were conducted in four replications in a randomized block design. The acaricides were applied at their recommended rates by a Stihl portable spraying device until run-off.

Biological efficacy against *P. ulmi* on apple was tested in a commercial orchard at Morović locality (45°00.711' N; 19°15.146' E), in plots of five trees. The efficacy against overwintering eggs of *P. ulmi* was investigated in trial F1. The acaricides were applied at the BBCH 09 growth stage (green leaf tips about 5 mm above bud scales) (Meier, 2001). Daily inspections of overwintering eggs in untreated plots showed that the treatment was applied five days before larval development. Motile forms of *P. ulmi* were counted on 50 young leaves per plot 12 days and 25 days after treatment. The efficacy against summer population was investigated in trials F2–F4. Motile forms were counted on 25 leaves per plot before treatment and three times after treatment.

The efficacy against *T. urticae* on cucumber was tested in a commercial greenhouse (trial GH) at Padinska Skela locality (44°57.012' N; 20°25.741' E) in plots of 10 plants. Motile forms were counted on ten leaves per plot (one whole leaf per plant) before treatment and twice after treatment.

TABLE 1. Field (F1–F4) and greenhouse (GH) trials against spider mites conducted in Serbia.

Pest	Trial	Date	Locality	Crop	Cultivar
Panonychus ulmi	F1	2004, 4/16–5/11	Morović	Apple	Red Chief
	F2	2004, 7/19–9/3	Morović	Apple	Granny Smith
	F3	2005, 7/27–11/12	Morović	Apple	Granny Smith
	F4	2008, 6/27–8/4	Morović	Apple	Red Chief
Tetranychus urticae	GH	2008, 8/12–8/23	Padinska Skela	Cucumber	Caman

TABLE 2. Pesticides applied in field (F1-F4) and greenhouse (GH) trials in Serbia.

Products	Active ingredients	Manufacturers	Trials
Talstar 10-EC	Bifenthrin (100 g L^{-1})	FMC International S.A., USA	F2, F3,
Fobos EW	Bifenthrin (100 g L^{-1})	Galenika-Fitofarmacija, Serbia	F4, GH
Apollo 50-SC	Clofentezine (500 g L^{-1})	Makhteshim Chemical Works, Israel	F1, F2, F3, F4,GH
Ortus 5-SC	Fenpyroximate (50 g L^{-1})	Nihon Nohyaku, Japan	F3
Demitan 200-SC	Fenazaquin (200 g L^{-1})	Margarita International, Portugal	F1
Envidor	Spirodiclofen (240 g L^{-1})	Bayer CropScience, Germany	F1, F2, F3, F4, GH

The numbers of motile forms of mites per plot were subjected to ANOVA and significant differences in means were identified by Tukey´s tests. The data were transformed by $\sqrt{X+0.5}$ before analysis. The efficacy of acaricides was calculated by Abbott's formula (1) in trial F1, and by Henderson-Tilton's formula (2) in trials F2–F4 and GH.

(1) $$Ef\ \% = (1 - N_t/N_c)\text{x}100$$

N = the number of motile forms per plot
t = treated plots
c = control plots

(2) $$Ef\ \% = [1 - (N_{ta}/N_{ca})(N_{cb}/N_{tb})]\text{x}100$$

N = the number of motile forms per plot
t = treated plots
c = control plots
a = after treatment
b = before treatment

Results and Discussion

Application of spirodiclofen (Envidor®) on overwintering *P*. *ulmi* eggs resulted in 89.1 and 86% efficacy in evaluations 12 and 25 days after treatment (DAT) (Table 3). Biological profile of spirodiclofen shows excellent activity against eggs and other immature stages of spider mites (Wachendorff *et al*., 2002; Bretschneider *et al*., 2007). Wachendorff *et al*. (2002) reported that spirodiclofen was more effective against mixed population of *P. ulmi* at 30°C than at 20°C, indicating a positive temperature coefficient. Our results indicate that spirodiclofen can be very effective even at relatively low temperatures (average daily temperature of 12°C). This effect of spirodiclofen is the result of its pronounced residual effect and good rain stability (Wachendorff *et al*., 2002). Biological efficacy of two other acaricides used for comparison was different: fenazaquin achieved efficacy close to that of spirodiclofen (91.3 and 84.4%, respectively), while clofentezine showed a considerably lower efficacy (67.4 and 27.8%). Such low efficacy of clofentezine, an ovo-larvicide which is recommended for application against overwintering eggs of *P. ulmi* (Aveyard *et al*., 1986), is probably a consequence of its long time usage at this locality.

TABLE 3. Numbers of *Panonychus ulmi* (means of motile forms per 25 leaves/plots) on apple 12 and 25 days after treatment (DAT) and efficacy (Ef%) of acaricides (trial F1).

Acaricides	g a.i.L-1	12 DAT	**Ef %**	25 DAT	**Ef %**
Spirodiclofen	0.096	1.5 c	**89.1**	3.8 b	**86.0**
Fenazaquin	0.1	1.2 c	**91.3**	4.2 b	**84.4**
Clofentezine	0.25	4.5 b	**67.4**	19.5 a	**27.8**
Untreated	-	13.8 a	**-**	27.0 a	**-**

Within a column, the means followed by different letters differ significantly (Tukey-test, $p< 0.05$).

In three trials of efficacy against the summer population of *P. ulmi* in three vegetation seasons (Tables 4–6), spirodiclofen considerably reduced population densities, maintaining them throughout the trial at a considerably lower level than the initial density. Spirodiclofen achieved high (91–98%) and long-lasting efficacy; in the third evaluation (38–47 DAT), the efficacy was equal to or somewhat higher than that recorded in the first evaluation (14–15

TABLE 4. Numbers of *Panonychus ulmi* (means of motile forms per 25 leaves/plots) on apple before treatment (BT), 15, 30 and 45 days after treatment (DAT) and efficacy (Ef%) of acaricides (trial F2).

Acaricides	g a.i.L^{-1}	BT	15 DAT	Ef %	30 DAT	Ef %	45 DAT	Ef %
Spirodiclofen	0.096	22.0 a	7.5 a	**91.0**	7.8 a	**97.2**	3.8 a	**98.0**
Clofentezine	0.3	37.2 a	44.2 b	**68.8**	43.3 b	**90.9**	127.2 b	**59.5**
Bifenthrin	0.02	58.8 b	48.8 b	**78.2**	179.5 c	**76.2**	188.8 c	**62.0**
Untreated	-	26.5 a	101.0 c	**-**	339.5 d	**-**	224.0 c	**-**

Within a column, the means followed by different letters differ significantly (Tukey-test, $p < 0.05$).

TABLE 5. Numbers of *Panonychus ulmi* (means of motile forms per 25 leaves/plots) on apple before treatment (BT), 14, 29 and 47 days after treatment (DAT) and efficacy (Ef%) of acaricides (trial F3).

Acaricides	g a.i.L^{-1}	BT	14 DAT	Ef %	29 DAT	Ef %	47 DAT	Ef %
Spirodiclofen	0.096	31.8 a	4.5 a	**95.2**	3.0 a	**96.3**	2.8 a	**95.7**
Fenpyroximate	0.025	26.5 a	2.0 a	**97.4**	1.8 a	**97.4**	27.0 bc	**50.3**
Clofentezine	0.3	17.2 a	22.0 b	**56.8**	10.0 b	**77.4**	13.2 b	**62.6**
Bifenthrin	0.02	24.0 a	24.2 b	**65.9**	42.5 bc	**31.1**	56.8 c	**0.0**
Untreated	-	23.8 a	70.5 c	**-**	61.2 c	**-**	48.8 c	**-**

Within a column, the means followed by different letters differ significantly (Tukey-test, $p < 0.05$).

TABLE 6. Number of *P. ulmi* [1)] on apple before treatment (BT), 14, 21 and 38 days after treatment (DAT) and efficacy (Ef%) of acaricides (trial F4).

Acaricides	g a.i.L^{-1}	BT	14 DAT	Ef %	21 DAT	Ef %	38 DAT	Ef %
Spirodiclofen	0.096	52.5 a	4.8 a	**97.4**	5.0 a	**95.6**	0.8 a	**97.0**
Clofentezine	0.3	42.2 a	75.0 b	**48.9**	29.2 b	**68.1**	13.0 b	**38.9**
Bifenthrin	0.02	47.5 a	193.0 c	**0.0**	126.0 c	**0.0**	87.0 c	**0.0**
Untreated	-	65.5 a	228.0 c	**-**	142.0 c	**-**	33.0 b	**-**

[1)] mean number of motile forms per 25 leaves/plot

Within a column, the means followed by different letters differ significantly (Tukey-test, $p < 0.05$).

DAT). In all three seasons, the acaricide was applied before an approximate damage threshold of five motile forms per leaf was reached. In trials carried out in several EU countries, spirodiclofen showed efficacy higher than 90% 26–32 DAT, when applied at the recommended concentration (0.096 g a.i.L^{-1}) against summer population of *P. ulmi* on apple trees at low to medium initial infestation (Elbert *et al*., 2002).

Clofentezine and bifenthrin showed unsatisfactory efficacy in the first season; in the next two seasons, their efficacy dropped to the level that practically made them useless against *P. ulmi* at the Morovic locality. This situation was probably the result of intense selection pressure on populations in the previous years. Use of clofentezine had been stopped before our first trial, while bifenthrin (and other pyrethroids) are still occasionally used against other pests. Although the resistance of this population still needs to be investigated, it is evident that spirodiclofen, an acaricide with a novel mode of action, is an excellent option to control *P. ulmi* at localities characterized by significantly reduced efficacy of other acaricides. In the second season trials, fenpyroximate demonstrated high efficacy (97.4%) in two initial evaluations, but in the third (47 DAT), its efficacy was almost twice lower. As an inhibitor of respiration, fenpyroximate can be used as an alternative to clofentezine and bifenthrin, although its activity is shorter in comparison to spirodiclofen. The rotation of a large number of acaricides with different mode of action is essential for implementation of an effective acaricide resistance management program (Wege & Leonard, 1994), as it extends the usage time of available acaricides and ensures effective control of European red mite in apple orchards.

Efficacy testing of spirodiclofen against *T. urticae* on greenhouse cucumber showed high effectiveness of this acaricide (Table 7). In evaluations 6 DAT and 10 DAT, spirodiclofen achieved 98.4 and 96.8% efficacy, while the efficacy of clofentezine was 95.4 and 93.4%, and efficacy of bifenthrin was 96.5 and 98.8%, respectively. Spirodiclofen is not registered for use against phytophagous mites on cucumber or any other greenhouse vegetable. As a highly polyphagous and cosmopolitan species, two-spotted spider mite is a common pest of greenhouse crops worldwide. The effective control of these populations is limited primarily by their extreme capability to develop acaricide resistance in a short time (van Leeuwen *et al*., 2009). Therefore, it is very important to expand the biochemical diversity of acaricides used against *T. urticae* by including spirodiclofen.

TABLE 7. Number of *T. uticae* [1)] on cucumber before treatment (BT), 6 and 10 days after treatment (DAT) and efficacy (Ef %) of acaricides (greenhouse trial).

Acaricides	g a.i.L^{-1}	BT	6 DAT	**Ef%**	10 DAT	**Ef%**
Spirodiclofen	0.096	14.4 a	0.2 a	**98.4**	0.3 a	**96.8**
Clofentezine	0.3	28.2 ab	1.1 a	**95.4**	1.2 a	**93.4**
Bifenthrin	0.05	26.9 ab	0.8 a	**96.5**	0.2 a	**98.8**
Untreated	-	21.4 ab	18.1 b	-	13.8 b	**-**

[1)] mean number of motile forms per leaf (1 whole leaf per plant, 10 plants per plot)
Within a column, the means followed by different letters differ significantly (Tukey-test, $p< 0.05$).

The results proved spirodiclofen to be effective in controlling European red mite on apple and two-spotted spider mite on cucumber, and a good alternative to older acaricides. The best application timing for this product is at the beginning of infestation and only one treatment per season or cropping cycle is recommended, in order to prevent or delay resistance development (Wachendorff *et al*., 2002; Elbert *et al*., 2002).

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