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Aspects on collateral effects of microbial insecticide preparations of *Bacillus thuringiensis* and other compounds on *Phytoseius finitimus* (Ribaga) (Parasitiformes Phytoseiidae)

Abstract - In two vineyards, variety "Rodhitis," located in the territories of Mesinikola near Karditsa and Nea Anhialos near Volos, have been carried out field trials to check collateral effects of six commercial preparations of microbial insecticide *Bt*, (Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP and Xen-Tari WG), two formulations of synthetic pyrethroid deltamethrin and two fungicides folpet and copper hydroxide (Kocide 101WP) on *Phytoseius finitimus* (Ribaga), during the years 2000-2002. The products were sprayed in the vineyard areas only, at the time recommended for the control of the grape moth and downy mildew respectively. The two formulations of the pyrethroid deltamethrin Decis EC and Decis WG (new formulation) were found high toxic (mortality 100% on juveniles and adults of *Ph. finitimus*) a week after application and its persistence lasted about for one month after spraying. The *Bt* preparations (Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP and Xen-Tari WG), appeared to be harmless one week after the first application. Ten days after the second application Agree WP, BPM 123 WP and Bactecin D showed slightly toxic, while Dipel 8L WP was moderately toxic (52.2%). However, twenty one days after the second application all the compounds were harmless afterwards. On the other hand, folpet and copper hydroxide were completely harmless on *Ph. finitimus*. These two fungicides and the *Bt* compounds may be used in itegrated control programs of and only the effect of Dipel 8L WP depends on its frequency of application .

Riassunto - *Effetti collaterali di preparati a base di* Bacillus thuringiensis *e di altri principi attivi su* Phytoseius finitimus *(Ribaga) (Parasitiformes Phytoseiidae).*

Negli anni 2000-2002, in due vigneti di varietà "Rodhitis" situati in Mesinikola (Karditsa) e Nea Anchialos (Volos), sono stati saggiati in pieno campo sei preparati comerciali dell'insetticida microbico *Bt*, (Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP e Xen-Tari WG), due formulazioni del piretroide sintetico deltametrina e due fungicidi, folpet e idrossido di rame (Kocide 101WP) per verificare gli effetti collaterali nei confronti di *Phytoseius finitimus*

(Ribaga). I prodotti utilizzati sono stati distribuiti nel momento raccomandato per la lotta contro le tignole e l'oidio. Le due formulazioni di deltametrina Decis EC e Decis WG (nuova formulazione), hanno provocato una mortalità del 100% nei confronti degli stadi giovanili e degli adulti di *Ph. finitimus* nella prima settimana dopo il trattamento; il loro effetto negativo si è protratto per quasi un mese. I preparati a base di *Bt* (Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP e Xen-Tari WG) sono risultati non tossici una settimana dopo la prima applicazione. Dieci giorni dopo il secondo trattamento Agree WP, BPM 123 WP e Bactecin D hanno evidenziato una leggera tossicità, mentre Dipel 8L WP è apparso moderatamente tossico (52,2%); 21 giorni dopo il secondo trattamento tutti i prodotti sono risultati di nuovo innocui. D'altra parte i fungicidi folpet e idrossido di rame sono risultati completamente innocui. Questi ultimi due prodotti ed i preparati a base di *Bt* potrebbero essere utilizzati in programmi di controllo integrato, mentre l'effetto di Dipel 8L WP dipende dalla frequenza di impiego.

Key words: collateral effects, *Bt* compounds, pesticides, *Phytoseius finitimus,* Phytoseiidae.

INTRODUCTION

It is known, that the species of the family Phytoseiidae are the most important group of natural enemies of phytophagous mites, especially of *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch) (Eveleigh & Chant, 1981; Sabelis, 1985; Dicke *et al.*, 1989; Liguori & Guidi, 1991; Papaioannou-Souliotis *et al.*, 1994, 1998, 1999; Markoyiannaki-Printzioy *et al.*, 2000). Twenty phytoseiid mites were found in continental and island Greek vineyards. *Phytoseius finitimus* (Ribaga) was seen to be particularly important in controlling the phytophagous mites (Papaioannou-Souliotis *et al.*, 1998, 1999).

The effect of insecticides, if the treatments are rarely carried out and with that active substances of a low persistence, on phytoseiid mites in viticulture is often considered less harmful compared with that of some fungicides. The toxicity of the insecticides used in the control of the grape moth *Lobesia botrana* (Denis & Schiffermueller) on phytoseiid populations has been tested (Dalla Monta' *et al.*, 1986; Duso *et al.*, 1988) and did not guarantee the survival of the phytoseiids mites. This phenomenon was more evident on the plants treated with organophosphorous and dithiocarbamates compounds (Duso, 1990; Redl & Fuchs, 1992; Solomon & Fitzgerald, 1993; Papaioannou-Souliotis *et al.*, 1998).

In I.P.C. programs the frequent use of preparations of microbial insecticide *Bacillus thuringiensis* and synthetic pyrethroids for the control of grape moth (in first, second and some times third generation) do not appear satisfactory for the density of phytoseiid populations (Dalla Monta' *et al.*, 1986; Petrova & Khrameeva, 1989; Erbach *et al.*, 1996; Papaioannou-Souliotis *et al.*, 1998).

So it is important to evaluate the toxicity of the six preparations microbial insecticides of *Bt*, the two synthetic pyrethroids (one new formulation) and the two fungicides for *Ph. finitimus,* since they are used more often in viticulture in our country. Furthermore, the available literature on this particular species in Greece as well as in the other European countries, where it has been recorded, is rather limited.

MATERIALS AND METHODS

The "Rodhithis" vineyards in the areas of Mesinikola near Karditsa and Nea Anhialos near Volos, where the tests were carried out from 2000 to 2002, were divided in 12 experimental plots (4 in N. Anhialos and 8 in Mesinikola).

In the experimental plots of Nea Anhialos were tested the fungicides Folpet 50% SC, Kocide 101 WP and the new formulation of the pyrethroid Decis WG which applied on June 03 and on September 08, 2000 respectively. In the experimental plots of Mesinikola were tested the formulation of the pyrethroid Decis EC on July 03, 2000 and the six preparations of microbial insecticide of *Bt* as Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP, Xen-Tari WG which applied on July 14 and July 23, 2000. Data on the formulations used and their recommended doses are listed in Table 1. In order to allow comparison of the effect of the above formulations on the populations of *Ph. finitimus*, the experimental plots were randomly subdivided in four groups (four repetitions) in each of which 5 vines were sampled. Each time 25 leaves were taken per group, resulting 25x4=100 leaves. The leaves were usually taken

Table 1 - Insecticides and fungicides used in vineyards of Mesinikola and Nea Anhialos during 2000-2002 season.

Commercial name	Active ingredients	Dose p.c./hl[1]
DECIS EC	deltamethrin	40 ml
DECIS EC	deltamethrin	60 ml
DECIS[2] WG	deltamethrin	10 gr
DECIS[2] WG	deltamethrin	20 gr
XENTARI WG	*B. t. aizawai*/15.000 IU/mg	100 gr
BACTOSPEINE WP	*B. t. kurstaki*/16.000 IU/mg	100 gr
AGREE WP	*B. t. strain* GC-91/25.000 IU/mg	100 gr
BPM 123 WP	*B. t. kurstaki*/32.000 IU/mg	50 gr
DIPEL 8L WP	*B. t. kurstaki*/32.000 IU/mg	50 gr
BACTECIN D	*B. t. kurstaki*/1.000 IU/mg	2 kgr/str (1str=1000m^2)
FOLPET 50% SC	folpet	250 ml
KOCIDE 101 WP	copper hydroxide	200 gr

[1] Doses are in accordance with company's instructions for use.
[2] New formulation

from the center piece of the shoots, at different points at approximately the same level. All treatments were applied with a conventional high-pressure spray motor and hand spray gun.

Sampling was done one day before spray and after that samples were taken at random, every other week. Leaves were collected in paper bags and placed in cool boxes and transferred to the laboratory. The predators were selected under stereomicroscope and killed in 70% alcohol. Subsequently they were cleared in Nesbitt's and mounted in Hoyer's liquid and identified under a phase contrast microscope.

Data were analyzed by standard ANOVA using the software package of Microsoft Excel-Windows 98 and mean differences were tested for significance using Duncan 's New Multiple Range Test (Duncan, 1955). Mortality rates were corrected for control mortality according to the formula of Abbott (Abbott, 1925).

RESULTS AND DISCUSSION

The populations of phytoseiid species found on the two vineyards in the sites Mesinikola (Karditsa) and Nea Anhialos (Volos), continued to consisted of *Ph. finitimus* (Papaioannou-Souliotis et al., 1999), which is the dominant predator amongst the five species were recorded. The *Ph. finitimus* populations reached level of 97.3% and 98.5% in 2000 and 2002 respectively of the total of phytoseiid mites found during our study. The species *Paraseiulus talbii* (Athias-Henriot) with 0.9%, *Typhlodromus hellenicus* Swirski & Ragusa with 0.8% and *Kampimodromus aberrans* (Oudemans) with 0.6% in Mesinikola and *T. hellenicus* with 0.9% and *Typhlodromus kerkirae* Swirski & Ragusa with 0.6% in Nea Anhialos showed low relative population densities. The presence of remaining species *Euseius finlandicus* (Oudemans), *Neoseiulus marginatus* (Wainstein), *Amblyseius andersoni* (Chant) in Mesinikola and *K. aberrans*, *P. talbii*, *Typhlodromus athiasae* Porath & Swirski and *Typhlodromus cotoneastri* Wainstein in Nea Anhialos can be considered incidental.

During the experiments, the effects of the two formulations of the synthetic pyrethroid deltamethrin, the six preparations of microbial insecticide *Bt* and the two fungicides on the survival and recovery of population of the *Ph. finitimus* were analyzed (Table 2).

The use of the two formulations deltamethrin Decis EC and Decis WG (new formulation) in high and low recommended doses caused drastic reductions in the density of *Ph. finitimus* populations in brief and slightly longer period of time after applications on July 03, 2000, with Decis EC and on September 08, 2000, with Decis WG. In the plots treated with Decis EC the numbers of *Ph. finitimus* remained very low, at both doses applied. This is shown even more clearly in Table 2, where the negative action rate is presented. Five days after application 100% mortality was recorded at both doses, while a month after treatment still any recovery observed. In the case of Decis WG is allowed a slightly recovery of the phytoseiid populations two weeks after application which continued till the last sampling date (mid November). As shown in Table 2, the numbers of *Ph. finitimus* remained significantly lower in comparison with

Table 2 - Results of the effects of insecticides and fungicides on the population of the predator Phytoseius finitimus *in the vineyards of Mesinikola and Nea Anhialos, during 2000-2002 season.*

Treatments	7.2.2002		7.7.02		7.20.02		8.6.02		8.19.02		9.8.02	
	Means[1] ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M
DecisEC 40 ml	69.5 ± 14.24 a[2]		0 b	100	0 b	100	0 b	100	0 b	100	1 ± 1.41 b	99.2
DecisEC 60 ml	71.5 ± 28.85 a		0 b	100	0 b	100	0 b	100	0.25 ± 0.5 b	99.5	3.25 ± 5.25 b	97.3
Control	80.5 ± 20.56 a		44.75 ± 2.75 a		71.25 ± 12.97a		80.75 ± 51.91 a		49 ± 21.77 a		121 ± 35.19 a	

Date of spraying: 7.3.2002

Treatments	9.7.2002		9.20.02		10.4.02		10.19.02		11.3.02		11.18.02	
	Means[1] ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M
*DecisWG 10 gr	200 ± 27.76 a[2]		3 ± 2.44 b	99.1	33 ± 8.83 b	93.6	55 ± 16.3 b	90.6	46 ± 5.09 b	89.9	50 ± 12.11 b	88.3
DecisWG 20 gr	231 ± 44.72 a		0 b	100	3 ± 1.63 b	99.4	19 ± 5.09 b	96.7	32± 8.04 b	92.9	37 ± 8.04 b	91.3
Control	247 ± 56.01 a		330 ± 31.08 a		520 ± 64.66 a		582.5 ± 68.05 a		457 ± 88.66 a		427 ± 48.42 a	

Date of spraying: 9.8.2002

* new formulation

Treatments	Before treatment		8 days after first treatment		10 days after the second treatment		21 days after the second treatment	
	Means[1] ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M
Xen-Tari	201 ± 22.61 a[2]		211.5 ± 18.62 a		168.5 ± 8.34 ab		164 ± 22.28 a	
Bactospeine	205.75 ± 26.72 a		189 ± 18.4 a		154.25 ± 44.34 ab		169.25 ± 23.54 a	
Agree	172.5 ± 21.64 a		166.25 ± 7.13 a		114.25 ±29.4 b	36.4	148.75 ± 26 a	
BMP	189.5 ± 8.01 a		182.25 ± 14.17 a		135.25 ± 21.76 b	24.9	171.5 ± 17.71 a	
Dipel	218 ± 76.22 a		213 ± 21.83 a		86 ± 10.86 c	52.2	188 ± 5.94 a	
Bactecin	221.5 ± 27.04 a		201.75 ± 30.37 a		96.5 ± 18.84 bc	46.4	182.25 ± 4.27 a	
Control	200.25 ± 48.16 a		201.25 ± 42.15 a		180 ± 32.16 a		168.75 ± 85.53 a	

Date of spraying: 7.14.2002, 7.23.2002

Treatments	6.2.2002		6.18.02		7.2.02		7.19.02		8.2.02	
	Means[1] ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M	Means ± SD	%M
Folpet	148.5 ± 36.46 a[2]		187.5 ± 21.01 a		199 ± 21.36 a		224 ± 84.92 a		187.5 ± 12.97 b	
Kocide	144.5 ± 17.33 a		202 ± 12.43 a		249.5 ± 55.78 a		180 ± 31.85 a		113.5 ± 18.08 a	
Control	186 ± 23.13 a		227.5 ± 38.31 a		212.5 ± 28,82 a		157 ± 42.11 a		90 ± 19.88 a	

Date of spraying: 6.3.2002

[1] Average of four repetitions of a sample of 25 leaves.

[2] Different letters in horizontal column denote significant difference (P<0.05, Duncan 's test).

the untreated, two months after treatment (88.3% and 91.3% rate mortality at the lower and higher dose respectively).

Evaluations of the influence of the above–mentioned insecticides on other important predators have given further evidence the negative effects of pyrethroids. Similar characteristics were found on populations of *Typhlodromus pyri* Scheuten on apple-orchards and grapevine. The formulation Decis EC was characterized by a less drastic effect than the other compounds (Hardman & Gaul, 1990; Kuijpers & Zwaan, 1992; Redl & Fushs, 1992; Gyorffyne, 1994; Block & Sengonca, 1996; Trapman & Boerties, 1999).

The effect of these insecticides, commonly used against grape moth in the I generation, on *Ph. finitimus* were shown to be highly toxic according to the scale of Hassan *et al.* (1994), even if only used in one year.

The influence of the treatments with the six formulations of microbial insecticide *Bt* (Agree WP, Bactecin D, Bactospeine WP, BPM 123 WP, Dipel 8L WP and Xen-Tari WG), for the control of grape moth, (commonly used in the II and maybe III generation) guarantees the survival of *Ph. finitimus* and therefore a successive recovery on the treated plots. This phenomenon was not observed on the plants treated with deltamethrin compounds.

Till September the population densities of the predator in the experimental plots appears normal, without significant fluctuations. After the third week a rapid growth of the population was observed as also reported by Papaioannou-Souliotis *et al.* (1998, 1999).

The evaluations of the influence of the above six preparations, on the development of *Ph. finitimus* is clear as shown in Table 2. Analysis of the data reveals no significant differences between any of the formulations applied and the control, eight days after the first application on July 14, 2000. However, the effect of four of the formulations after their second application on July 23, 2000 is notable. After the second treatment a slight difference is observed in the population densities of *Ph. finitimus* treated with Dipel 8L WP, Bactecin D, BPM 123 WP and Agree WP as compared to the other two formulations Xen-Tari WG, Bactospeine WP and the control. Ten days after the second application (08.02.2000) a slight drop in population of the predator is apparent for the Agree WP (36.4% mortality), BPM 123 WP (24.9%) and Bactospeine WP (46.4%), while for Dipel 8L WP (52.2%) has to be considered moderate mortality (Table 2). These data confirm once more that the moderate toxicity of Dipel 8L WP on population of *Ph. finitimus* depends on the mode and frequency of application, alone or mixed with other compounds (Papaioannou-Souliotis *et al.*, 1998). Thus, according to the toxicity scale by Hassan *et al.* (1994), Dipel 8L WP when applied once classifies in scale I (non toxic), but when it is applied repeatedly during the same growing season and at short intervals it is classified in scale II (moderate toxicity), while Bactecin D and BPM 123 WP are classified in scale II for the first week after the second application. Three weeks after the second application they return again in scale I (non toxic), meaning that they are considered harmless as all other formulations of microbial insecticide *Bt*.

In agriculture the considerations and possibilities involved in using *Bt* in I.P.C. programs are complicated (Navon, 2000). Many studies have aimed at evaluating the effects of this microbial insecticide with its preparations. Thus, Chapman & Hoy (1991), observed that the strain *tenebrionis* at recommended doses causes high mortality in *Galedromus occidentalis* (Nesbitt) and low in *T. urticae*. The strain *galleriae*, in combination with cypermethrin 0.5%, causes high mortality in the predators *Neoseiulus fallacis* (Garman) and *Phytoseiulus persimilis* Athias-Henriot but without insecticide the mortality drops to 1.7% and 7.1% respectively (Dong & Niu, 1991). The strain *kurstaki* is characterized as moderate toxic to *T. pyri* on grapevine and apple-orchards at recommended doses (Hardman & Gaul, 1990; Vogt, 1995; Erbach *et al.*, 1996). The strain *thuringiensis* was reported to be very harmful to *P. persimilis* (Petrova & Khrameeva, 1989). It also reduced the fertility when this predator were sprayed directly, while it was less toxic when applied to the leaves previously. On the other hand, on populations of *T. pyri* it was evaluated low toxic (Haas, 1987) and non toxic on apple-orchards (Niemczyk, 1997). Similar results were carried out on the predators *A. andersoni* and *Euseius stipulatus* (Athias-Henriot) (Strapazzon *et al.*, 1986; Niemczyk, 1997), while this preparation on the *Typhlodromus exhilaratus* Ragusa in grapevine was evaluated non toxic (Liguori, 1988).

The applications with fungicides folpet and copper hydroxide (Kocide 101WP) against downy mildew did not seem to effect the population of *Ph. finitimus*. The predator *Ph. finitimus* developed normally after application on June 03, 2000 according to the season and in July the density of predator get over the control. The evaluations of the influence of the above mentioned have given further evidence in Table 2. Both compounds are non toxic to *Ph. finitimus* and classify in scale I according to Hassan *et al.* (1994), (Duso *et al.*, 1988; Papaioannou-Souliotis *et al.,* 1998).

Finally, the results of this study together with the previous surveys and experiments have been carried out for over four years on biological control of phytophagous mites in these areas, considered of great importance for viticulture, gave fundamentals elements to the management of phytoseiid mites, especially *Ph. finitimus*, and constituted the guidelines in the future for the application of integrated pest control in a large area of Central Greece.

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