

Compatibility of *Nesidiocoris tenuis* and *Iphiseius degenerans* with insecticides, miticides and fungicides used in tomato crops

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Abstract

Nesidiocoris tenuis (Reuter) (Heteroptera Miridae) and *Iphiseius degenerans* (Berlese) (Acari Phytoseiidae) are omnivorous predators commonly used in IPM in Europe. The aim of this work was to assess the side effects of eight insecticides and miticides and four fungicides widely applied in IPM on each predator species. Lethal toxicity trials were performed on sprayed and unsprayed nymphs of *N. tenuis* and unsprayed females of *I. degenerans* when exposed to freshly dried residues of the pesticides used at the label doses. Sub-lethal effects (oviposition rate) were recorded on survived females of *I. degenerans*. As food, untreated eggs of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) or pollen were offered to *N. tenuis* or *I. degenerans* individuals, respectively. The effects were monitored for a period up to 10 days after treatment. Exposure to imidacloprid and deltamethrin residues were harmful for the sprayed nymphs of *N. tenuis* but moderately and slightly harmful for the unsprayed ones, respectively. Fludioxonil + cyprodinil was classified as moderately harmful and abamectin, etoxazole, fosetyl-Al, pyraclostrobin + boscalid as slightly harmful, at both ways of exposure. Finally, pymetrozine, methoxyfenozide, spiromesifen, bifenazate and azoxystrobin were classified as harmless in both application scenarios. In the case of *I. degenerans*, imidacloprid, deltamethrin, abamectin, etoxazole and bifenazate were classified as harmful, spiromesifen, fosetyl-Al, pyraclostrobin + boscalid and fludioxonil + cyprodinil as slightly harmful and pymetrozine, methoxyfenozide and azoxystrobin were classified as harmless. The positive control (chlorpyrifos-methyl) caused 100% mortality 1 day after treatment and the negative control (distilled water) caused no mortality in both predators. Pymetrozine and methoxyfenozide did not cause any significant adverse effect on the number of eggs laid by *I. degenerans* females. Azoxystrobin caused a highly negative effect although it had no acute lethal effects. In the cases of deltamethrin and abamectin delayed mortality response of *N. tenuis* appeared indicating that the use of an extended observation period is useful for accurate evaluation of the pesticide side effects. Taken together, the current study's outcomes advance more effective and sustainable approaches in the compatibility of pesticides and the use of these beneficial arthropods in IPM, clearly indicating that the side effects of fungicides should not be ignored.

Key words: non-target effects, side effects, sub-lethal effects, mortality, IPM, Miridae, Phytoseiidae.

Introduction

Nesidiocoris tenuis (Reuter) (Heteroptera Miridae) is an omnivorous predator widely used in biological control programs against *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae), whiteflies, and other pests in tomato crops (Perdikis *et al.*, 2011; Chailleux *et al.*, 2013; Itou *et al.*, 2013; Hassanpour *et al.*, 2016; Biondi *et al.*, 2018). The prevention of incompatibility between *N. tenuis* and pesticides' use is a major challenge in IPM implementation in tomato crops against the insect pest *T. absoluta*. This is because extensive use of insecticides may disrupt biological control that ultimately results in failures in the control of this devastating pest (Desneux *et al.*, 2007; Urbaneja *et al.*, 2012; Martinou and Stavrinos, 2015). In addition, this kind of knowledge will be essential in application of IPM strategies in the control of *T. absoluta* in the new countries it has invaded since *N. tenuis* occurs in most of them, including Asian and African countries, Central and North America (Zappalà *et al.*, 2013; Ballal *et al.*, 2016; Campos *et al.*, 2017; van Lenteren *et al.*, 2018; CABI, 2019; Ferracini *et al.*, 2019; Han *et al.*, 2019).

The side effects of pesticides on *N. tenuis* have been reported for emamectin benzoate, azadirachtin, spinosad and indoxacarb (Arnó *et al.*, 2011; Lopez *et al.*, 2011),

flonicamid (Roditakis *et al.*, 2014), flubendiamide, spirotetramat, metaflumizone and sulfoxaflor (Wanumen *et al.*, 2016) pyriproxyfen, spirotetramat, cypermethrin (Ziaei Madbouni *et al.*, 2017), sulphur (Zappalà *et al.*, 2012) and a citrus essential oil based biopesticide (Soares *et al.*, 2019). These studies offer information but knowledge on the impact of many other commonly used pesticides on this predator is still lacking. Additionally, *N. tenuis* populations either released or naturally colonizing tomato crops are exposed to a potentially high risk of fungicides' adverse effects due to the relatively long period required for their establishment and their long persistence on the crop. In fact, fungicides are usually applied repeatedly, particularly in the period of fast plant growth which coincides with the period of *N. tenuis* release and establishment (Dáder *et al.*, 2019). In addition, the release of *N. tenuis* in the nursery (pre-plant release method) has shown promising results in accelerating its establishment in the tomato crops but in nurseries fungicides are routinely applied (Calvo *et al.*, 2012a; 2012b; Perdikis *et al.*, 2015; Madeira *et al.*, 2019). In this respect, fungicides may strongly affect the predator's efficacy in IPM. In a recent study by Ziaei Madbouni *et al.* (2017) the application of seven fungicides (benomyl, chlorothalonil, copper oxychloride, cyazofamid, fluopicolide + propamocarb hydrochloride, penconazol, trifloxystrobin) was considered

compatible with *N. tenuis*. Wettable sulphur was proved harmless towards *N. tenuis* while dustable sulphur resulted in being moderately harmful as a fresh residue and slightly harmful as a 7-day-old residue; no effects were recorded exposing the predator to 14-day-old sulphur residues (Zappalà *et al.*, 2012). However, there is a wide range of fungicides commonly used in tomato crops and thus further studies should focus on the susceptibility of *N. tenuis* to them.

Another aspect that merits attention is the potential of *N. tenuis* to produce damage to tomato crops when it occurs with high numbers in the period following the elimination of prey populations. Although this is dependent on its population densities (Sánchez and Lacasa, 2008; Perdakis *et al.*, 2009; Castañé *et al.*, 2011; Siscaro *et al.*, 2019), a rational management of its population may become necessary. Thus, the assessment of side effects of pesticides may potentially indicate which of them could be applied to assist in its control when deemed necessary within IPM programs.

A major group of natural enemies used against the two-spotted spider mite, thrips and whiteflies in greenhouse crops is the phytoseiid predators (Heinz *et al.*, 2004). Due to their common use for several decades, the side effects of pesticides have been studied in many phytoseiid species in vegetable crops (Kim *et al.*, 2006; 2018; Bostanian *et al.*, 2009; Colomer *et al.*, 2011; Cuthbertson *et al.*, 2012; Kaplan *et al.*, 2012; Döker *et al.*, 2015; Ditillo *et al.*, 2016). However, the different phytoseiid species may have a different response to chemicals and thus species-specific studies must be conducted (Irigaray and Zalom, 2006). Even more, literature search revealed that our knowledge on the non-target effects of fungicides on phytoseiid mites in tomato crops remains at a relatively low level (Angeli and Ioriatti, 1994; Blumel *et al.*, 2000; Auger *et al.*, 2004; Ditillo *et al.*, 2016).

Iphiseius degenerans (Berlese) (Acari Phytoseiidae) is a generalist predator feeding on mites, pollen and thrips on several host plants occurring in Europe, Asia and Africa (McMurtry and Croft, 1997; van Rijn and Tanigoshi, 1999; Fantinou *et al.*, 2012; Tsolakis *et al.*, 2016). This predator is commercialized and used against the western flower thrips *Frankliniella occidentalis* (Pergande) and the two spotted spider mite *Tetranychus urticae* Koch in greenhouse crops (Vantornhout *et al.*, 2004; Chow *et al.* 2008; Tsolakis *et al.*, 2016). Non-target effects of pesticides on this mite have been reported (Brown, 2005; van Driesche *et al.*, 2006; Döker *et al.*, 2015) but knowledge on the effects of fungicides is limited (Cuthbertson *et al.*, 2012).

The aims of this work were to investigate the non-target effects of insecticides, miticides and fungicides commonly used in tomato crops, on both *N. tenuis* and *I. degenerans*. To address this goal, their side effects were evaluated on *N. tenuis* by exposing nymphs to two exposure routes a) untreated nymphs to freshly dried residues and b) multiple exposure scenario, i.e. exposing treated nymphs to freshly dried residues. In the case of *I. degenerans* females were exposed to freshly dried residues and effects on their survival and egg laying were recorded. In addition, a prolonged experimental period was followed for a proper assessment of the effects of chemicals on natural enemies (Stara *et al.*, 2011).

Materials and methods

Pesticides and fungicides

The products used were 4 insecticides, 2 miticides, 2 with both insecticidal and miticidal properties and 4 fungicides, all of them commonly used in tomato crops (table 1). All compounds were evaluated by applying their maximum field recommended concentrations for tomato crops, according to the product dose labels and following the Phytosanitary Products Registry of the Hellenic Republic Ministry of Rural Development and Food. Chlorpyrifos-methyl was used as positive control based on its well-known adverse effects on non-target organisms. Distilled water was used as negative control and for preparation of the spraying solutions.

Culture of the study organisms

Rearing of *N. tenuis* was initiated from nymphs and adults purchased from Koppert Biological Systems, The Netherlands (NesibugTM), and kept on tomato plants (cv. Elpida F1, Spirou House of Agriculture, Athens, Greece) with eggs of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) (Koppert Biological Systems) as prey. The rearing of *I. degenerans* was initiated from individuals collected on citrus in Aitolokarnania region in Western Greece. The rearings were conducted in arenas of Plexiglas (26.5 × 15 × 5 cm) according to Fantinou *et al.* (2012). Twice a week *Prunus amygdalis* Batsch pollen was added. The cultures of both predators were kept at 25 ± 1 °C, 65 ± 5% RH and 16L:8D photoperiod.

Experimental setup

The side effects of the pesticides on the survival of *N. tenuis* nymphs were evaluated following two routes of exposure: 1) unsprayed nymphs exposed to freshly dried residues on tomato leaves and 2) multiple exposure (sprayed nymphs foraging on leaves with freshly dried residues). Nymphs were used instead of adults because they are flightless and most likely more exposed to the toxic effects of the pesticides. This approach represents a pragmatic, worst-case scenario, which, however, commonly occurs in agricultural practice due to the long persistence of the predator in the crops.

Tomato plants, each with six to seven fully developed leaves (ca. 40 cm in height) were sprayed using a hand trigger sprayer. The nozzle of the sprayer was set to mist position and wetting all the leaves of the plant and its stem uniformly until runoff. The plants were shade dried for one hour. Then, one 3rd instar nymph of the predator was singly placed on the second or the third fully developed leaf of the plant, counting from the top. This procedure followed because *N. tenuis* is mostly present on the upper part of the tomato plants (Perdakis *et al.*, 2014). On that leaf untreated eggs of *E. kuehniella* were added as a factitious prey offered *ad libitum* (>200 eggs). Following that, the leaf with the nymph and the prey eggs was carefully enclosed in a muslin cage (15 × 20 cm). To ascertain the effects of multiple exposure the same procedure was followed but the nymph was placed on the leaf before the spraying application.

After the enclosure of the nymphs, the plants were transferred to 25 ± 1 °C, 65 ± 5% RH and 16L:8D photo-

Table 1. Details of the pesticides (IRAC, 2018; FRAC, 2018) and their concentration used in the bioassays of the current study.

Active ingredient (a.i.)	Commercial name - manufacturer	Mode of action	Chemical family	Target pests on product label	a.i./L spraying solution
I n s e c t i c i d e s / m i t i c i d e s					
Imidacloprid	Confidor Forte 200 SL - Bayer CropScience, Germany	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Neonicotinoids	Whiteflies	100 mg L ⁻¹
Deltamethrin	Decis 2,5 EC - Bayer CropScience, Germany	Sodium channel modulator	Pyrethroids, Pyrethrins	Several insect pests	12.5 mg L ⁻¹
Pymetrozine	Plenum 50 WG - Syngenta Crop Protection, UK	Chordotonal Organ TRPV Channel Modulators	Pyridine azomethine derivatives	Whiteflies and aphids	300 mg L ⁻¹
Chlorpyrifos methyl	Reldan 225 EC - Dow Agrosciences, UK	Acetylcholinesterase (AChE) inhibitors	Organophosphates	Several insect pests	675 mg L ⁻¹
Methoxyfenozide	Runner 240 SC - Bayer CropScience, Germany	Ecdysone receptor agonists	Diacylhydrazines	<i>Spodoptera</i> spp.	96 mg L ⁻¹
Abamectin	Vertimec 1,8 EC - Syngenta Crop Protection, UK	Glutamate-gated chloride channel (GluCl) allosteric modulators	Avermectins, Milbemycins	<i>Tetranychus</i> spp., <i>Liriomyza</i> spp. and Thripidae	14.4 mg L ⁻¹
Spiromesifen	Oberon 240 SC - Bayer CropScience, Germany	Inhibitors of acetyl CoA carboxylase	Tetronic and tetramic acid derivatives	Whiteflies and <i>Tetranychus</i> spp.	144 mg L ⁻¹
Etoxazole	Borneo 11 SC - Sumitomo Chemical, Japan	Mite growth inhibitors	Etoxazole	<i>Tetranychus</i> spp.	38 mg L ⁻¹
Bifenazate	Floramite 240 SC - Chemtura, The Netherlands	Mitochondrial complex III electron transport inhibitors	Bifenazate	Mites	96 mg L ⁻¹
F u n g i c i d e s					
Fosetyl-Al	Aliette 80 WG - Bayer CropScience, Germany	Unknown mode of action	Ethyl phosphonates	<i>Phytophthora infestans</i>	3.2 g L ⁻¹
Azoxystrobin	Ortiva 25 SC - Syngenta Crop Protection, UK	Respiration: complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)	Methoxy-acrylates	<i>Leveillula taurica</i> , <i>Oidium</i> sp., <i>P. infestans</i> , <i>Alternaria dauci</i> sp. <i>solani</i>	200 mg L ⁻¹
Pyraclostrobin + boscalid	Signum 26,7/6,7 WG - Basf Crop Protection, USA	Respiration: complex II: succinate-dehydro-genase Complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)	Etylphosphonates Methoxy-carbamates	<i>Botrytis cinerea</i> , <i>Leveillula taurica</i>	Boscalid 400 mg L ⁻¹ Pyraclostrobin 100.5 mg L ⁻¹
Fludioxonil + cyprodinil	Switch 25/37,5 WG - Syngenta Crop Protection, UK	Signal transduction: MAP/Histidine- Kinase in osmotic signal transduction Amino acids and protein synthesis: methionine biosynthesis (proposed)	Phenylpyrroles Anilino-pyrimidines	<i>Botrytis</i> spp. <i>Sclerotinia</i> spp.	Fludioxonil 250 mg L ⁻¹ Cyprodinil 375 mg L ⁻¹

period. The mortality of the nymphs was assessed 1, 2, 3, 5, 7 and 10 days after treatment (DAT). Prey eggs were added on the leaf with the predator at each inspection. Each treatment was replicated 10 times with one nymph being one replicate. The control treatments were applied following the same methodology as the test compounds. A tested individual was considered dead if no movement was recorded after being prodded with a fine bristle.

In the case of *I. degenerans* the experiments were conducted in plastic Petri dishes of 4.5 cm diameter and 1.5 cm high. On their lid a round hole was opened that was covered with muslin to reduce the level of humidity. In the experiments, 1-2 days old females were used. In this assay, the base of the formerly described Petri dish was sprayed with each substance and then was left to dry for 1 hour. Then, a moistened cotton layer was added in the periphery of the base of the dish and one unsprayed female was released at the centre of the dish. In the dish, almond pollen was added at the beginning of the experiment that was replaced by fresh pollen 5 days later. The dish was wrapped with paraffin tape. The survival of each female was recorded 1, 2, 3, 5, 7, and 10 DAT. Simultaneously, sub-lethal effects were assessed by counting any eggs laid. The eggs were removed at each inspection. Ten replicates per treatment were used. In the very few cases (2 in total) that females became entangled in the cotton the replications were discarded and were replaced by new ones.

Statistical analysis

The effects of the compounds on the mortality of the tested individuals were evaluated with the Log-Rank procedure used to compare the respective survival curves. Using the data on oviposition rates of *I. degenerans* females the mean number of eggs laid per day for the period that each female was alive was calculated and the data analysed with one-way ANOVA after log transformation. The means were separated by the Tukey-Kramer HSD test ($\alpha = 0.05$). Statistical analyses were performed with the statistical package JMP (SAS Institute, 2016).

The mortality rates were scored following the standard classification of International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC): Pesticides that cause mortality <30% were classified as harmless, pesticides that cause mortality from 30 to 79% as slightly harmful and from 80 to 99% as moderately harmful; products that cause mortality >99% were classified as harmful (Sterk *et al.*, 1999).

Results

Nesidiocoris tenuis

The mortality caused by each pesticide on nymphs of *N. tenuis* after residual or multiple exposure is shown in figures 1A and 1B, respectively. In this figure the results of the comparisons of the respective survival curves per treatment are shown, too. In the residual effects, chlorpyrifos-methyl had the most adverse effect, inflicting 100% mortality of *N. tenuis* (figure 1A). The survival curve of this insecticide did not differ significantly to that of imidacloprid (90% total mortality), fludioxonil +

cyprodinil (90%) and deltamethrin (70%). The effect of deltamethrin was higher but not significantly different to these of abamectin, etoxazole, fosetyl-Al and pyraclostrobin + boscalid. Pymetrozine, methoxyfenozide, spiromesifen, bifentazate and azoxystrobin did not induce significant mortality in comparison to the control (water) where no nymph was found dead.

The mortality rates per observation of *N. tenuis* showed that when chlorpyrifos-methyl was used all nymphs died by the first day of exposure (figure 1A). In the case of imidacloprid the mortality was 50% on the third day after treatment (3DAT) and increased to 80% at 5DAT and finally to 90% at the last record (10DAT). Mortality from deltamethrin ultimately reached 70%, but the first dead individuals (10%) appeared only five days after the initiation of the experiment whereas most dead individuals (50%) were recorded 10DAT. In the case of abamectin mortality rate of 20% was recorded 5DAT and again after 10DAT. After applying fosetyl-Al mortality first appeared 3DAT (10%) and increased to 20% 5DAT and finally to 40% at the last record (10DAT). After the application of fludioxonil + cyprodinil 30% of the nymphs died at 2DAT, another 30% died at 5DAT but mortality continued to increase and 10DAT reached to 90%.

When multiple exposure effects were assessed, the mortality attained its highest value (100%) after imidacloprid, deltamethrin or chlorpyrifos-methyl applications (figure 1B). Their survival curves were not significantly different to those of abamectin and fludioxonil + cyprodinil (70% and 80% mortality, respectively). The mortality rates of abamectin and fludioxonil + cyprodinil were followed by those inflicted by fosetyl-Al and pyraclostrobin + boscalid, the survival curves of which were not different to each other. Pymetrozine, methoxyfenozide, spiromesifen, etoxazole, bifentazate had a slight effect, whereas no nymph was found dead after the application of azoxystrobin. In these experiments no nymph survived already on the first day when chlorpyrifos-methyl but also when imidacloprid was applied (figure 1B). It should be noted that deltamethrin caused 50% mortality already on the first day, which remained almost constant till 7DAT when increased to 90%. Mortality due to abamectin attained 30% 1DAT and an additional 30% 2DAT and subsequently changed only on the 10th day, finally increasing to 70%. Methoxyfenozide, spiromesifen, and etoxazole caused mortality only 10DAT reaching to 20%, 10% and 30%, respectively. Fosetyl-Al had an adverse effect 1DAT (20%) that increased by 10% 2DAT and 10DAT. Fludioxonil + cyprodinil caused mortality within 3DAT (70%) that was deteriorated further 10DAT (10%).

The comparison of the survival curves between the residual and multiple exposure bioassays showed significant differences for imidacloprid and deltamethrin, where a stronger adverse effect was recorded under the multiple exposure scenario ($\chi^2 = 19$, d.f. = 1, $P < 0.05$, and $\chi^2 = 10.92$, d.f. = 1, $P < 0.05$, respectively).

According to the IOBC classification (Sterk *et al.*, 1999), imidacloprid and deltamethrin were classified as harmful when the nymph was sprayed (table 2). However, the effect of imidacloprid was moderately harmful and that of deltamethrin slightly harmful when the nymph

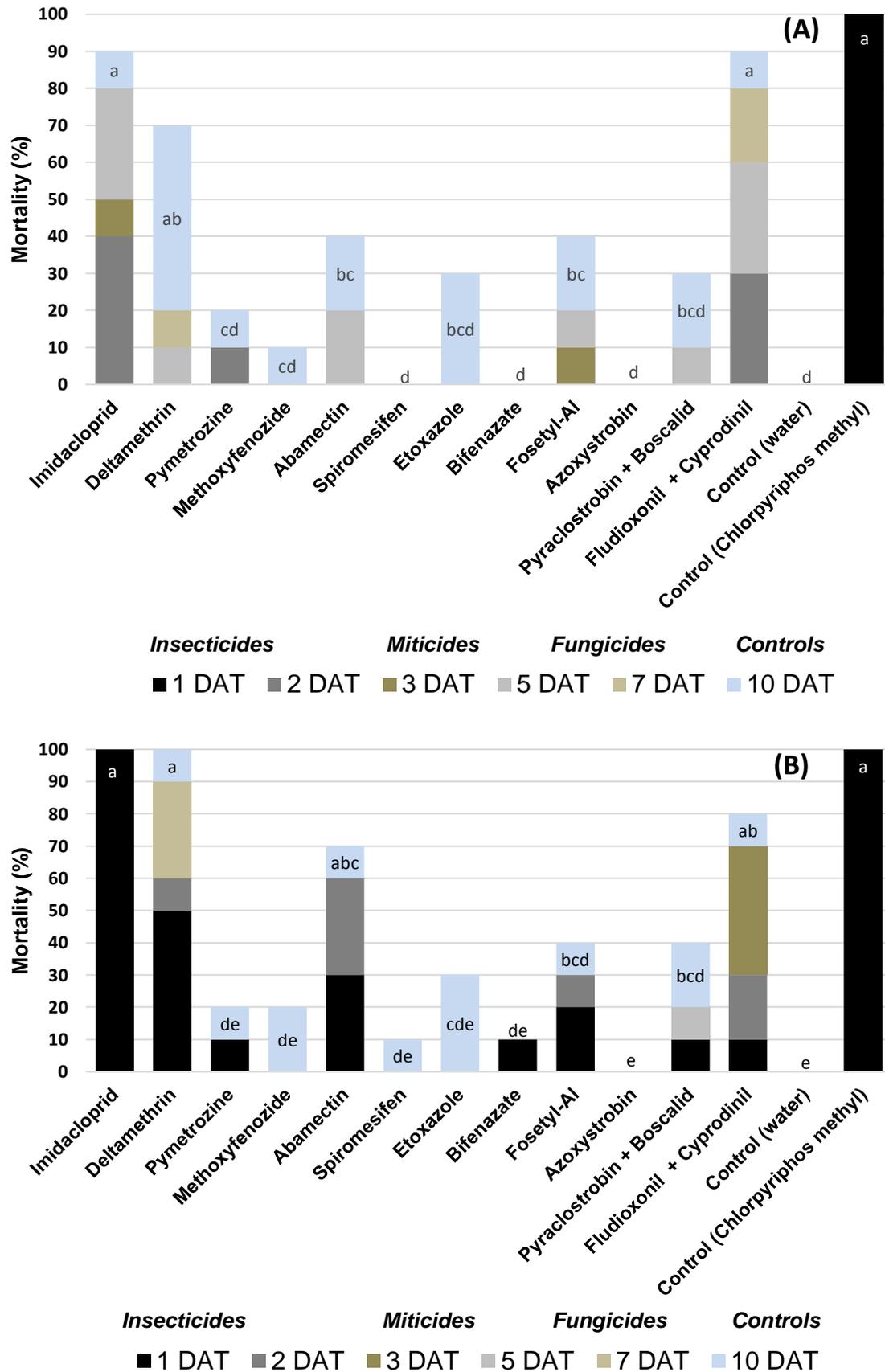


Figure 1. Percentage mortality caused by insecticides, miticides and fungicides on *N. tenax* after 1, 2, 3, 5, 7 and 10 days of exposure of unsprayed nymphs to freshly dried residues (A) and sprayed nymphs to freshly dried residues (B). Columns sharing the same letter indicate no significant differences in the respective survival curves among treatments ($P > 0.05$, Log-Rank procedure).

Table 2. Classification of pesticides tested in the current study according to the IOBC categories.

Active ingredient	Trade name	IOBC Classification ¹		
		<i>Nesidiocoris tenuis</i> Multiple exposure	<i>Nesidiocoris tenuis</i> Residual effect	<i>Iphiseius degenerans</i> Residual effect
Insecticides/miticides				
Imidacloprid	Confidor	4	3	4
Deltamethrin	Decis	4	2	4
Pymetrozine	Plenum	1	1	1
Methoxyfenozide	Runner	1	1	1
Abamectin	Vertimec	2	2	4
Spiromesifen	Oberon	1	1	2
Etoxazole	Borneo	2	2	4
Bifenazate	Floramite	1	1	4
Fungicides				
Fosetyl-AI	Aliette	2	2	2
Azoxystrobin	Ortiva	1	1	1
Pyraclostrobin + boscalid	Signum	2	2	2
Fludioxonil + cyprodinil	Switch	3	3	2

¹ IOBC categories: 1 - harmless (reduction <30%); 2 - slightly harmful (30-79%); 3 - moderately harmful (80-99%); 4 - harmful (>99%) (Sterk *et al.*, 1999).

was not sprayed. Fludioxonil + cyprodinil showed in both cases a moderately harmful effect. Abamectin, etoxazole, fosetyl-AI and pyraclostrobin + boscalid at both ways of exposure were slightly harmful. Finally, pymetrozine, methoxyfenozide, spiromesifen, bifenazate and azoxystrobin were classified as harmless in both application scenarios.

Iphiseius degenerans

Lethal effects

The mortality of the females of this phytoseiid reached 100% when imidacloprid, deltamethrin, abamectin, etoxazole, bifenazate or chlorpyrifos-methyl had been used. Among them, deltamethrin, abamectin and chlorpyrifos-methyl survival curves significantly differ to those of imidacloprid and bifenazate but not to that of etoxazole. Significantly lower mortality levels were caused by spiromesifen (60%) and pyraclostrobin + boscalid (60%), fludioxonil + cyprodinil (40%) and fosetyl-AI 30% without differing significantly to each other (figure 2). Mortality levels caused by pymetrozine and methoxyfenozide were lower, without differing to the previously mentioned ones and the control. Azoxystrobin did not cause any mortality.

Deltamethrin, abamectin and chlorpyrifos-methyl caused 100% mortality already 1DAT. Mortality caused by etoxazole or bifenazate reached to 90% 2DAT. Imidacloprid caused a mortality of 30% 2DAT that was gradually increased to 100% 7DAT. Mortality caused by spiromesifen appeared 3DAT (30%) and increased to 50% on the 5th day and finally to 60% 10DAT. Pyraclostrobin + boscalid caused 30% 5DAT that reached to 60% 10DAT. Mortality caused by fludioxonil + cyprodinil was 40% and appeared 5DAT.

Following the IOBC classification (Sterk *et al.*, 1999), imidacloprid, deltamethrin, abamectin, etoxazole and bifenazate were classified as harmful, spiromesifen, fosetyl-AI, pyraclostrobin + boscalid and fludioxonil +

cyprodinil as slightly harmful and pymetrozine, methoxyfenozide and azoxystrobin were classified as harmless.

Sub-lethal effects

The average number of eggs laid per female of *I. degenerans* was dependent on the treatment ($F_{13,126} = 30.64$, $P < 0.001$) (figure 3). The number of eggs laid by females exposed to residues of pymetrozine or methoxyfenozide was not significantly different to that of the negative control treatment where the highest value was recorded. These values were followed by significantly lower ones recorded after the application of spiromesifen, azoxystrobin, fludioxonil+cyprodinil and fosetyl-AI, which were not significantly different to each other. A further but not significant reduction in the number of eggs laid were recorded following the application of imidacloprid and pyraclostrobin+ boscalid. Finally, a significantly reduced or zero number of eggs recorded for deltamethrin, abamectin, etoxazole and bifenazate that was not significantly different to that recorded after the application of the positive control.

Discussion

Nesidiocoris tenuis

Among the insecticides tested, imidacloprid was harmful after multiple exposure and moderately harmful after residual exposure. Consequently, imidacloprid is not compatible with release or conservation of *N. tenuis*. Highly adverse effects on adults of *N. tenuis* have been reported by Kim *et al.* (2018) (79.4% mortality after topical application of imidacloprid, 2DAT). Deltamethrin was classified as harmful after multiple exposure. The toxicity of imidacloprid and deltamethrin was found different between the two routes of exposure used, being greater when the nymphs were sprayed. Similar results are reported by Ziaei Madbouni *et al.* (2019) who showed

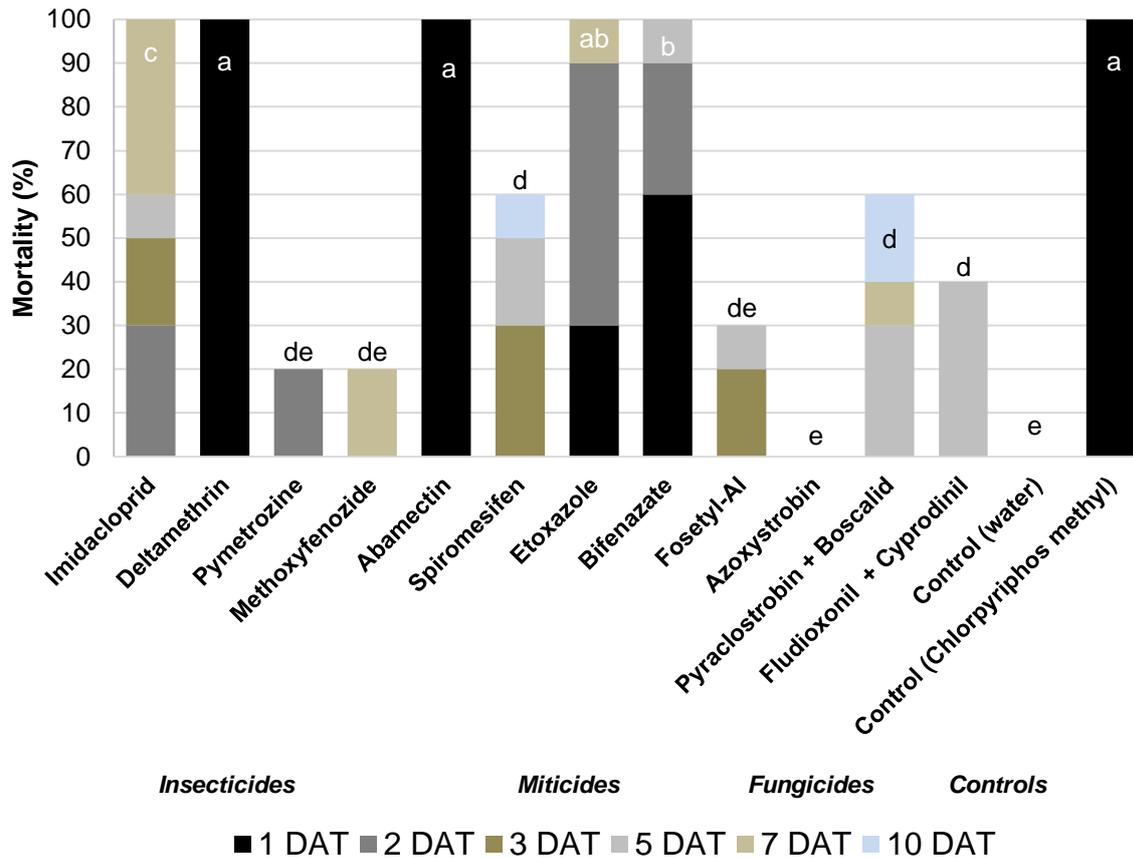


Figure 2. Percentage mortality caused by insecticides, miticides and fungicides on females of *I. degenerans* after 1, 2, 3, 5, 7 and 10 days of their exposure to freshly dried residues. Columns sharing the same letter indicate no significant differences in the respective survival curves among treatments ($P > 0.05$, Log-Rank procedure).

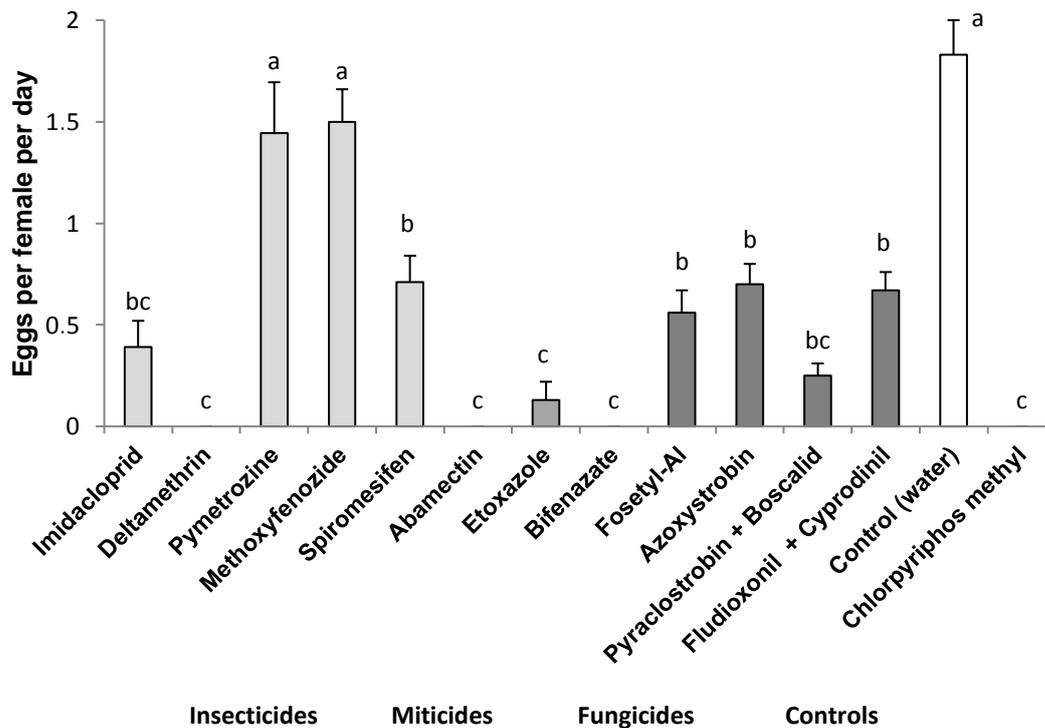


Figure 3. Fecundity (mean number of eggs laid per female \pm SE) of *I. degenerans* females during 10 days from their exposure to freshly dried residues of insecticides, miticides and fungicides and controls. Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Tukey test.

that cypermethrin caused 98% mortality to *N. tenuis* adults after multiple exposure whereas mortality was negligible when exposed to dry residues. Residual and topical applications of deltamethrin caused similar toxic effects on larvae of *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae) (Pathan *et al.*, 2008). However, deltamethrin effects were worse when topically sprayed on adults of *Apis mellifera* L. (Hymenoptera Apidae) (Costa *et al.*, 2014) and imidacloprid effects were more severe when topically applied on the green apple aphid (Lowery and Smirle, 2003). Thus, studies compiling both residue and topical exposure can offer information in the most appropriate use of insecticides under IPM programs.

The insecticides pymetrozine and methoxyfenozide were classified as harmless to *N. tenuis* irrespectively of the exposure method. Methoxyfenozide had very little toxic effect on nymphs and adults of *Deraeocoris brevis* (Uhler) (Heteroptera Miridae) (Kim *et al.*, 2006). The miticide spiromesifen was classified as harmless. This substance had little effect on adults of *N. tenuis* 48 hours after topical application (Kim *et al.*, 2018).

Abamectin had a significant effect on the nymphs of *N. tenuis* classified as slightly harmful. Abamectin is known to have a quick knockdown effect against insect pests (Gharalari *et al.*, 2009) and this was also observed here, where the sprayed individuals died at a percentage of 60% already in 2DAT.

Etoxazole was classified as slightly harmful to *N. tenuis*. Its effects have not been searched for this or another heteropteran predator. Bifenazate was harmless for the nymphs of *N. tenuis*. Similarly, this substance was proved safe to the nymphs of *Pilophorus typicus* (Distant) (Heteroptera Miridae) (Nakahira *et al.*, 2010).

The fungicide fludioxonil + cyprodinil was classified as moderately harmful to *N. tenuis* in both application scenarios. However, there is no information on the effects of this fungicide on *N. tenuis*. Fludioxonil + boscalid caused no mortality on adults of *N. tenuis* 48h after application (Kim *et al.*, 2018) whereas boscalid had no significant effect on the nymphs and adults of *P. typicus* (Nakahira *et al.*, 2010). Therefore, likely cyprodinil may be responsible for the adverse effects recorded in the current study on the survival of *N. tenuis*. Further in the present work, azoxystrobin was classified as harmless but the mixture of pyraclostrobin + boscalid was slightly harmful in both routes of exposure used. In another work, azoxystrobin was also classified as harmless for the predatory bug *Orius insidiosus* (Say) (Hemiptera Anthracoridae) (Andorno *et al.*, 2019). However, trifloxystrobin was found to cause a significant mortality on *N. tenuis* adults (28%) in the multiple exposure scenario after 7DAT (Ziaei Madbouni *et al.*, 2017). In our study, fosetyl-Al was found always to cause a significant adverse effect on *N. tenuis* and was classified as slightly harmful. Thus, the fungicide fludioxonil + cyprodinil is not compatible with the release or conservation strategies of *N. tenuis*, whereas pyraclostrobin + boscalid and fosetyl-Al should be carefully used after the release of *N. tenuis* in tomato crops or in tomato crops naturally colonized by *N. tenuis*. Further research should focus on specific investigations of the impacts of cyprodinil, pyraclostrobin and fosetyl-Al on *N. tenuis*.

In the current study, the mortality of *N. tenuis* nymphs was assessed up to 10 DAT. This extended observation period enabled us to record mortality that appeared several days after treatment. In the residual exposure experiments, this was informative for deltamethrin lethal effects where the first dead individuals appeared only in the 5DAT and much increased 10DAT. Similar results were recorded for the residual effects of abamectin where the first dead individuals recorded 5DAT and a similar mortality was recorded 10DAT, as well as in the multiple exposure bioassay for deltamethrin where mortality of 50% on the first day, increased on the 5th day and finally reached to 100% 10DAT. In the residual exposure experiments methoxyfenozide and etoxazole and in the multiple exposure experiments methoxyfenozide, spiromesifen and etoxazole caused mortality only 10DAT. Therefore, our results indicate that the monitoring period in this kind of experiments should be long enough for a more accurate assessment of the residual toxic effects of pesticides on natural enemies. This kind of evidence should be considered in research efforts to identify the safe distance between the last spraying and the release of a natural enemy.

Iphiseius degenerans

The insecticides imidacloprid and deltamethrin were classified as harmful to *I. degenerans*. The effect of imidacloprid was found to be detrimental on the survival of *Phytoseiulus persimilis* Athias-Henriot (Acari Phytoseiidae) (Sterk *et al.*, 2002). However, in another study imidacloprid was not lethal to *P. persimilis* (Dittilo *et al.*, 2016). Its effects on *Euseius gallicus* Kreiter et Tixier (Acari Phytoseiidae) were classified as moderately harmful (Put *et al.*, 2016). Imidacloprid and pyrethrin caused high mortality in adults of *Neoseiulus californicus* (McGregor) (Acari Phytoseiidae) (Castagnoli *et al.*, 2005). Among 4 phytoseiid species, thiocloprid, an insecticide with the same mode of action as imidacloprid had significant adverse effects only on *I. degenerans* that caused 60% and 70% mortality after indirect and direct treatments (Cuthbertson *et al.*, 2012). Therefore, the different phytoseiid species may differ in their responses but *I. degenerans* seems to be a sensitive species to imidacloprid and deltamethrin.

The influence of pymetrozine on *I. degenerans* was not significant. Similar results were obtained for the same species (30% mortality after 48 hours of topical exposure) (Cuthbertson *et al.*, 2012). Slight effects have also been reported for other phytoseiids (James *et al.*, 2002; Sechser *et al.*, 2002; Castagnoli *et al.*, 2005).

Methoxyfenozide had no significant effect on *I. degenerans*. This insecticide has been reported as compatible with the release of *Amblyseius swirskii* Athias-Henriot (Acari Phytoseiidae) since its population remained almost at similar levels 30 days after the treatment of a pepper crop (Colomer *et al.*, 2011).

Abamectin was highly toxic to *I. degenerans*. In another study, 24 hours after its exposure to dried residues, the mortality rate of *I. degenerans* already reached 90% (Cuthbertson *et al.*, 2012). Abamectin also has been reported to be highly toxic to *P. persimilis* and *N. californicus* (Kaplan *et al.*, 2012) and on *E. gallicus* (Put *et al.*, 2016).

Spiromesifen was slightly harmful to *I. degenerans*. This substance has been evaluated for other phytoseiids and its effects vary with the species under study. Its toxic effects were more harmful in *P. persimilis* than *N. californicus* (37% and 5% 24 hours after the application, respectively) (Cloyd *et al.*, 2006) whereas this substance significantly reduced the adult survival of *Galendromus occidentalis* (Nesbitt) (Acari Phytoseiidae) (Irigaray and Zalom, 2006).

Etoxazole was classified as harmful. This substance caused a very slight effect on the survival of the predatory mite *G. occidentalis* (Irigaray *et al.*, 2006) and a slight harmful effect on *P. persimilis* and *Amblyseius womersleyi* Schicha (Acari Phytoseiidae) (Kim and Seo, 2001; Kim and Yoo, 2002). Generally, this compound is considered to be safe for beneficials (Dekeyser, 2005). However, our results contrast with those of the above-mentioned studies (Kim and Seo, 2001; Kim and Yoo, 2002; Dekeyser, 2005; Irigaray *et al.*, 2006) likely indicating the higher sensitivity of *I. degenerans* to etoxazole in comparison to other phytoseiid species.

Bifenazate was also proved to be harmful to *I. degenerans*. However, a series of studies indicate that its effects differ among the various phytoseiid species tested. The application of bifenazate on females of three phytoseiid species caused mortality rates ranging between 57.3% and 80.8% (James, 2002). A mortality rate of 7% for *N. californicus* and 67% for *P. persimilis* 24 hours after their exposure to dried residues has been reported (Cloyd *et al.*, 2006). This substance did not influence the adult survival of *G. occidentalis* and *P. persimilis* (Kim and Yoo, 2002; Irigaray and Zalom, 2006). This, together with the results of etoxazole support the suggestion that separate investigations should be conducted for each phytoseiid species (Put *et al.*, 2016).

The fungicide fosetyl-Al was slightly harmful. There is not much information on its side effects on phytoseiids. However, it has also been considered as slightly harmful to phytoseiids on fruit crops (Fountain and Med, 2015). Azoxystrobin had no significant effect on the survival of the females of *I. degenerans*. According to Silva *et al.* (2019) azoxystrobin showed low toxicity to *N. californicus* whereas it had no significant effect on the survival of *P. persimilis* (Duso *et al.*, 2008; Dittilo *et al.*, 2016). Fludioxonil + cyprodinil was slightly harmful. Information on its effects on other phytoseiids came only for *E. galli-cus* showing that it was harmless (Put *et al.*, 2016).

Pyraclostrobin + boscalid was classified as slightly harmful to *I. degenerans*. The effects of boscalid on the mortality and fecundity of *G. occidentalis* and *P. persimilis* were non-significant (Bernand *et al.*, 2004; Dittilo *et al.*, 2016). Strobilurins such as pyraclostrobin have been reported to be innocuous to predacious mites (Alston and Thomson, 2004; Laurin and Bostanian, 2007). Boscalid and pyraclostrobin were examined separately on *G. occidentalis* and did not cause a significant effect (Bostanian *et al.*, 2009). This difference from our results could be due to the different species but also to the mixture (pyraclostrobin+ boscalid) that was used, and further research should follow to clarify their effects.

The substances that were highly toxic, accordingly caused detrimental effects on mite fecundity. An adverse

effect was shown for azoxystrobin. In this case, although mortality rates were 0% during 10DAT, the number of eggs laid was 62% reduced in comparison to the control. Fosetyl-Al caused a highly adverse effect on fecundity although it was found to be slightly harmful. Therefore, sub-lethal effects were significant, supporting that studies on sub-lethal effects should accompany studies on non-target effects (Desneux *et al.*, 2007; Fernandes *et al.*, 2016). Thus, the evaluation of both toxic and sub-lethal effects offer important insights in the compatibility of these two substances with *I. degenerans*. There was no effect of pymetrozine and methoxyfenozide on the number of eggs laid by *I. degenerans*. This confirms their compatibility with *I. degenerans*.

In conclusion, the current paper offers insights into the toxic effects of commonly used insecticides and miticides as well as of fungicides, useful to assess their compatibility with the preservation or release of *N. tenuis* and *I. degenerans*. Among the substances tested, compatible with *N. tenuis* are the insecticides pymetrozine and methoxyfenozide, the miticides spiromesifen and bifentazate and the fungicide azoxystrobin. Abamectin and etoxazole but also the fungicides fosetyl-Al and pyraclostrobin + boscalid were ranked as slightly harmful and should be used with caution. Deltamethrin is not compatible when applied after the release of the predator and it was moderately harmful when applied before the predator's release whereas its toxic effects appeared after a relatively long period post-treatment. This is important information for IPM practitioners who should apply deltamethrin much before the predators are released. Imidacloprid and the fungicide fludioxonil + cyprodinil can be considered as being not compatible, either when applied before or after the release of the predator. Further research on the effects of the studied pesticides on the demographic parameters or the predatory behaviour of *N. tenuis* (Soares *et al.*, 2019) may offer insights in their compatibility with the release of *N. tenuis*.

In the case of *I. degenerans*, the insecticides pymetrozine and methoxyfenozide had a highly selective profile whereas fosetyl-Al and fludioxonil + cyprodinil is recommended to be used with caution. Spiromesifen was slightly harmful, whereas abamectin, etoxazole and bifentazate were harmful. Azoxystrobin was not toxic but caused a highly adverse effect on the fecundity, thus more research is required to clarify its compatibility with the use of this predator. Overall, the present study delivered valuable results for the successful integration of insecticides, miticides and fungicides and biological control in IPM. Further experimentation under more field-related conditions will be useful to fully evaluate the non-target effects of these chemicals on the beneficials *N. tenuis* and *I. degenerans*.

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Received November 27, 2019. Accepted May 4, 2020.