Chemosphere 96 (2014) 167–173

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Lethal and behavioral effects of pesticides on the insect predator *Macrolophus pygmaeus*

A.F. Martinou [a], N. Seraphides [b], M.C. Stavrinides [a,*]

[a] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Arch. Kyprianos 30, Limassol 3036, Cyprus
[b] Agricultural Research Institute, Nicosia 1516, Cyprus

HIGHLIGHTS

- Safety of seven pesticides to the insect predator *M. pygmaeus* was evaluated.
- Chlorantraniliprole, an anthranilic diamide, caused the lowest mortality.
- Thiacloprid, a neonicotinoid, caused the highest mortality.
- Thiacloprid induced stronger behavioral effects than chlorantraniliprole.

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 28 September 2013
Accepted 5 October 2013
Available online 5 November 2013

Keywords:
Conservation biological control
Mirid predator
Toxicity
Time allocation

ABSTRACT

Macrolophus pygmaeus (Hemiptera: Miridae) is a common generalist predator in Mediterranean agroecosystems. We evaluated the lethal effects of six insecticides and a fungicide on *M. pygmaeus* nymphs exposed to the pesticides through three routes of exposure: direct, residual and oral. Chlorantraniliprole and emamectin-benzoate caused less than 25% mortality to *M. pygmaeus* and were classified as harmless according to the International Organization for Biological Control rating scheme. In contrast, thiacloprid and metaflumizone caused 100% and 80% mortality, respectively, and were classified as harmful. Indoxacarb and spinosad resulted in close to 30% mortality to the predator, and were classified as slightly harmful, while the fungicide copper hydroxide caused 58% mortality and was rated as moderately harmful. Chlorantraniliprole and thiacloprid were selected for further sublethal testing by exposing *M. pygmaeus* to two routes of pesticide intake: pesticide residues and feeding on sprayed food. Thiacloprid led to an increase in resting and preening time of the predator, and a decrease in plant feeding. Chlorantraniliprole resulted in a decrease in plant feeding, but no other behaviors were affected. In addition, thiacloprid significantly reduced the predation rate of *M. pygmaeus*, whereas chlorantraniliprole had no significant effect on predation rate. The results of the study suggest that thiacloprid is not compatible with *M. pygmaeus*, while further research needs to be carried out for metaflumizone and copper hydroxide. All other products seem to be relatively compatible with *M. pygmaeus*, though studies on their sublethal effects will shed more light into their safety.

1. Introduction

Native natural enemies such as predators and parasitoids provide the essential ecosystem service of conservation biological control (Gardiner et al., 2011). The use of agrochemicals, particularly pesticides, can hamper the effectiveness of natural enemies, causing disruption of the ecosystem services they provide (Desneux et al., 2007; Stark et al., 2007; Stavrinides and Mills, 2009). Natural enemies are highly sensitive to the application of pesticides, and it has been shown that pesticide use patterns influence the abundance and species composition of beneficial species in agro-ecosystems (Zhang et al., 2007; Lu et al., 2012).

The evaluation of pesticides for registration purposes and compatibility with IPM programs traditionally begins with an assessment of their acute toxicity that can provide important information on the risk they pose to natural enemies (Candolfi et al., 2001). The importance of sublethal pesticide effects on development and reproduction of predators and parasitoids has also been recognized by many researchers, including Croft (1990), Desneux et al. (2007), Biondi et al. (2012a) and Pekár (2012). Although publications on behavioral effects of pesticides on natural enemies have increased in recent years (e.g. Desneux et al., 2004; Delpuech et al., 2012; Wrinn et al., 2012), there is still a lot to be learnt about the behavioral impacts of pesticides on predators and parasitoids.

* Corresponding author. Tel.: +357 25 002186; fax: +357 25 002834.
E-mail address: m.stavrinides@cut.ac.cy (M.C. Stavrinides).

0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.10.024

An important behavioral aspect of natural enemies that has not been studied extensively is time allocation following pesticide exposure (Desneux et al., 2004) i.e. the time invested to different activities, such as prey searching, feeding and resting. Time allocation has been a central issue in behavioral ecology over the past few decades, because the amount of time allocated to different activities by a natural enemy can influence its fitness and its effectiveness as a biological control agent (Bernstein et al., 1988; Yano et al., 2005; van Laerhoven et al., 2006).

Various factors have been found to determine the amount of foraging time that natural enemies spend on different activities, including leaf surface characteristics, the number of prey available, the prey distribution, the presence of prey traces and plant leaf damage (Dixon, 2000; van Laerhoven et al., 2000, 2006; Nakashima and Hirose, 2003). Studies on how chemicals affect time allocation and the foraging success of beneficial arthropods could be integrated in the evaluation of pesticides during registration procedures (Desneux et al., 2007).

In this study, we evaluated lethal and behavioral effects of pesticides to the predator *Macrolophus pygmaeus* (Hemiptera: Miridae). The predator is native to the Mediterranean region and it has also been commercially mass produced and successfully released in temperate and Mediterranean crops including tomato and other vegetables (Martinou and Wright, 2009). *M. pygmaeus* is polyphagous and can survive in the absence of prey by feeding on plant sap (Lykouressis et al., 2008). It is being marketed for use against whiteflies, thrips, aphids, mites, and eggs and larvae of lepidopterous pests (Urbaneja et al., 2009, 2012; Perdikis et al., 2011). It is one of a few native natural enemies that have been shown to be effective against the tomato borer, *Tuta absoluta* (Lepidoptera: Gelechidae) (Desneux et al., 2010), a major invasive pest of tomato that invaded Europe through Spain in 2006 and continues to spread in Afro-Eurasia (Desneux et al., 2011). Several insecticides are being used against *T. absoluta* in Europe, but their effects on *M. pygmaeus* are little known. Two recent studies tested a limited number of products for their safety to *M. pygmaeus*, by exposing insects to pesticide residues on plants (Arnó and Gabarra, 2011; Lopez et al., 2011), a methodology not accounting for pesticide intake through direct application on the body of the insect, and through feeding on treated prey. Exposing individuals to three routes of pesticide exposure represents a realistic, worst-case scenario, and a very common case in agricultural fields.

Our aims in this project were (a) to evaluate the lethal effects of seven pesticides registered for use in tomato crops to *M. pygmaeus* by simultaneously exposing individuals to direct application, residues and sprayed food, and (b) to identify the effects of thiacloprid and chlorantraniliprole, two insecticides representing different modes of action and toxicity levels, on time allocation and predation rate of *M. pygmaeus*.

2. Materials and methods

2.1. Pesticides

Seven pesticides were tested: the synthetic insecticides thiacloprid, metaflumizone, indoxacarb and chlorantraniliprole, the microorganism-derived insecticides spinosad and emamectin benzoate, and the natural fungicide copper hydroxide (Table 1). The selected insecticides are used against the invasive tomato borer, *T. absoluta*, and other important tomato pests, such as whiteflies (Table 1). Application of the six insecticides in tomato crops has seen a dramatic increase in Cyprus and other Mediterranean countries since 2006, when *T. absoluta* was reported on the European continent for the first time. Copper hydroxide is a product that is regularly applied on tomato crops for the control of downy mildew, but its effects on *M. pygmaeus* are not known.

2.2. Lethal effects of pesticides

M. pygmaeus nymphs were exposed to pesticides through a triple exposure method: directly through contact with spray droplets, orally through their food, and residually through walking on spayed plant leaves. A triple exposure scenario represents best the conditions that insects are likely to experience in the field following pesticide application.

M. pygmaeus fifth-instar nymphs and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs provided by Koppert (Netherlands) were used for the experiments. Fifth-instar nymphs were used because they cannot fly and therefore they are more exposed to pesticide applications than flying adults. The insects were provided with tomato leaves and *E. kuehniella* eggs as food and water for 24 h before being used in experiments. A Potter spray tower (Burkard Manufacturing Co., Rickmansworth, UK) was used for the spray application on tomato leaflets, the predators and the egg prey, at the highest label rates (Table 1). The air pressure was set at 1000 Kpa. The spray volume per application was 1 mL of pesticide solution which resulted in a spray deposit of 2.55 mg cm^{-2} similar to what is recommended for bioassays according to the IOBC Working Group "Pesticides and Beneficial Organisms" (Hassan et al., 2000).

Tomato leaflets were cut from 6 to 8 week-old tomato plants variety Hybrid Brillante F1 (Hazera Genetics Ltd., 79837, Israel) and they were enclosed in net envelopes (mesh aperture size 0.54 mm^2) in sets of five. Both sides of the leaflets were sprayed

Table 1
Pesticides used in the study. All pesticides were used at their highest label rate for tomato crops.

Active ingredient (a.i.)	Commercial name-manufacturer	Chemical family	Mode of action	Target pests on product label	Highest label rate mg a.i./L
Chlorantraniliprole	CORAGEN®-DuPont Crop Protection	Anthranilic diamide	Ryanodine receptor modulator	Lepidoptera	40.00
Copper hydroxide	CHAMP 37.5 WG®-Nufarm	Metallic hydroxide	Fungal protein disruptor	Downy mildew and several other fungi	1925.00
Emamectin benzoate	AFFIRM®-Syngenta	Avermectin	Chloride channel activator	Lepidoptera	14.25
Indoxacarb	STEWARD EC®-DuPont Crop protection	Oxadiazine	Voltage dependent sodium channel blocker	Lepidoptera	37.50
Metaflumizone	ALVERDE 24SC®-BASF	Semicarbazone	Voltage dependent sodium channel blocker	Lepidoptera	240.00
Spinosad	TRACER 480 EC®-Dow AgroSciences	Spinosyn	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Thrips, Lepidoptera, Coleoptera, Diptera	72.00
Thiacloprid	CALYPSO 480 SC®-Bayer CropScience	Chloronicotinoid	Nicotinic acetylcholine receptor agonist	Lepidoptera, Hemiptera (aphids and *Psylla* spp.)	144.00

with 1 mL of pesticide solution or distilled water for the control in the Potter spray tower. The leaflets were allowed to dry and they were placed individually in a transparent plastic container (4 cm height, 2.5 cm diam.). The petiole of the leaflet was in contact with moist cotton wool through a circular opening at the base of the plastic container.

Nymphs of *M. pygmaeus* were placed in 9 cm Petri dishes in groups of fifteen and they were sprayed with 1 mL of each pesticide solution or distilled water for the control. Similarly, *E. kuehniella* eggs were placed in a 9 cm Petri dish and sprayed with pesticide solution or distilled water for the control. *M. pygmaeus* nymphs and *E. kuehniella* eggs were allowed to dry, and the predators were transferred individually to the containers containing the leaflets with an abundance of sprayed eggs as prey. The top of the container was sealed with fine muslin and a rubber band. Insects were kept individually as preliminary studies showed cannibalism incidents when predators were kept in the same container. Each treatment was replicated five times, with fifteen *M. pygmaeus* individuals per replicate. The insects were kept at 25 ± 2 °C, 16:8 L:D and 65% RH. Mortality assessments were carried out at 72 h. Moribund individuals were classified as dead. Because of the large number of treatments and number of insects tested, the experiment was carried out in two sets, on two consecutive days. On day 1 we run assays for chlorantraniliprole, spinosad, metaflumizone and control and on day 2 we run assays for indoxacarb, copper hydroxide, thiacloprid and emamectin benzoate with a second control.

Pesticides were classified into classes following IOBC guidelines (Sterk et al., 1999). Pesticides that cause mortality up to 25% are classified as harmless, pesticides that cause mortality from 25 to 50% are classified as slightly harmful and from 51 to 75% as moderately harmful. Harmful are the products that cause mortality over 75%.

2.3. Behavioral effects of pesticides

In a second assay, we evaluated the sublethal effects of thiacloprid and chlorantraniliprole, two key products that represent different modes of action, and different toxicity levels (Fig. 1). Thiacloprid is a neonicotinoid insecticide that acts as agonist on the insect nicotinic acetylcholine receptor of the nervous system, and has been widely used in tomato pest management (Elbert et al., 2008). Chlorantraniliprole is a newer product, an anthranilic diamide that activates the ryanodine receptor releasing stored calcium from muscle cells that leads to impaired regulation of muscle contraction (Cordova et al., 2006). Thiacloprid and chlorantraniliprole caused the highest and lowest mortality in the lethal effects assay (Fig. 1). Because thiacloprid caused 100% mortality to *M. pygmaeus* (Fig. 1), we modified our approach and included only two routes of pesticide exposure: Residual via treated leaf surfaces and oral via treated food. The two routes of pesticide exposure simulated a scenario where the predator escaped direct contact with spray droplets and was later exposed to the pesticide via walking on treated surfaces and consuming treated egg prey or plant material. Preliminary experiments showed that *M. pygmaeus* exposed to thiacloprid through sprayed leaf surfaces and treated food suffered 50% mortality at 72 h, while chlorantraniliprole mortality was similar to control.

Fifth instar *M. pygmaeus* nymphs were collected one day prior to the experiment and placed individually in Petri dishes (9 cm diameter). A tomato leaflet and a piece of wet cotton wool were placed in each Petri dish. The predators were not sprayed with pesticide.

Tomato seedlings (five weeks old, height 15 cm, 5 leaves/plant) were sprayed till run off with a handheld sprayer (Di Martino type Venezia 500, Mussolente, Italy) with pesticide or distilled water for the control. *E. kuehniella* eggs were placed in a 9 cm Petri dish and were also sprayed with pesticide or distilled water till run-off using the handheld sprayer. The plants and the eggs were allowed to dry and two hours later, we transferred 15 sprayed *E. kuehniella* eggs on the first leaflet of the first true leaf of the tomato plant using a fine paintbrush. A *M. pygmaeus* nymph was placed at the bottom of the main stem of the plant with the aid of a moist paint brush. The nymph was observed for 11 min with a 2× magnifying glass held at a 45° angle and at a 20 cm distance. The duration for each of the following behaviors was recorded: walking, time to arrival at the egg patch, feeding on the egg patch, plant feeding, preening and resting. For data recording we used a program designed in qbasic for MS-DOS (Martinou et al., 2009). The experiment was carried out over seven days between 10:00 and 14:00 with three replicates of each treatment per day.

After the end of the 11 min interval, the tomato seedlings were placed in transparent plastic containers (diameter: 15 cm, height: 25 cm) which were sealed on the top with fine muslin for ventilation. Prey consumption was estimated 24 h later by counting the number of consumed *E. kuehniella* eggs. *M. pygmaeus* has piercing-sucking mouthparts and consumed eggs appear shrivelled. The number of dead *M. pygmaeus* in each treatment were recorded

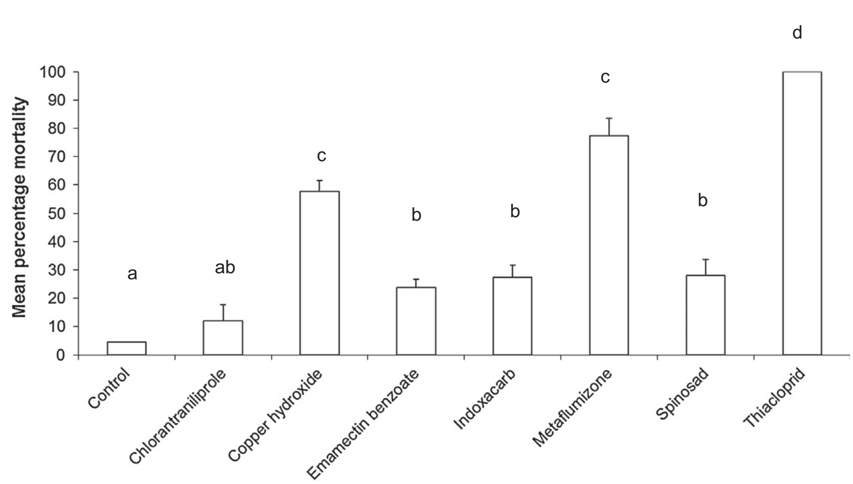

Fig. 1. Percentage mortality (mean ± 1SE) caused by different pesticides to fifth-instar nymphs of *M. pygmaeus* at 72 h after exposure. Different letters indicate statistically significant differences ($P < 0.05$, one-way ANOVA followed by the Tukey multiple comparison test).

at 24 and at 72 h. Each of the two pesticide treatments and the control were replicated 21 times, and were kept at the same conditions as for the experiments on lethal effects of pesticides (Section 2.2).

2.4. Statistical analyses

Lethal effects of pesticides: We compared percentage mortality in controls for day 1 and day 2 using a *t*-test (*t*. test) in R (version 2.14.2, R Development Core Team, 2006). As there were no significant differences in control mortality between experiments carried out on day 1 and day 2 (see Results section), we grouped the data for the two days and evaluated the effects of pesticides on percentage mortality in a one-way analysis of variance (ANOVA) (aov). Post hoc Tukey HSD tests (TukeyHSD) were used for multiple pairwise comparisons. Before analyses, all data were transformed using the arcsine square root transformation to achieve normality and homoscedasticity of variance.

Behavioral effects of pesticides: behavioral data were expressed as time in seconds allocated to each different activity over the 11 min observation period. An ANOVA model (aov) was used to compare the time allocated to each activity in the different treatments in R 2.14.2. An ANOVA was also used to compare the egg consumption rate at 24 h among treatments. Data on consumption rate were transformed using the arcsine square root transformation to achieve normality and homoscedasticity of variance. Dead individuals were excluded from the egg consumption rate analysis. Post hoc Tukey HSD tests (TukeyHSD) were used for multiple pairwise comparisons.

3. Results

3.1. Lethal effects of pesticides

There were no significant differences in control mortality between experiments carried out on day 1 and day 2 ($t_8 = 0.64$, $P > 0.05$). The ANOVA results showed significant differences in *M. pygmaeus* mortality among different treatments ($F_{7.37} = 69.6$, $P < 0.001$) (Fig. 1). Thiacloprid caused 100% mortality to *M. pygmaeus*, while chlorantraniliprole was the least toxic product (Fig. 1), and the mortality caused by chlorantraniliprole did not differ significantly from control mortality. Metaflumizone and copper hydroxide caused the second and third highest mortality, respectively, while indoxacarb, spinosad, and emamectin benzoate caused similarly low mortality, close to 30%.

3.2. Behavioral effects of pesticides

Fig. 2 presents the time allocated to four main activities: Resting, preening, feeding from the plant and walking. No predators arrived at the egg patch during the 11 min observation period. There were significant differences in resting time among the control and the two pesticide treatments ($F_{2.60} = 7.39$, $P < 0.001$). *M. pygmaeus* spent a significantly lower amount of time resting on control plants compared to the thiacloprid treatment. Significant differences were also found in the amount of time spent preening ($F_{2.60} = 3.4$, $P < 0.05$). *M. pygmaeus* on control plants spent a lower amount of time preening, compared to plants sprayed with thiacloprid. There were also significant differences in the amount of time spent feeding from the plant ($F_{2.60} = 17$, $P < 0.001$). *M. pygmaeus* on control plants spent fed significantly longer time feeding from the plant than insects on plants sprayed with either pesticide. Total walking time did not differ significantly ($F_{2.60} = 2.52$, $P = 0.08$) among treatments and control.

The predators did not consume eggs of *E. kuehniella* during the 11 min observation period, but 24 h later there were statistically significant differences in the number of consumed eggs between control and treatments ($F_{2.48} = 6.268$, $P < 0.001$, Fig. 3). The percentage of eggs consumed was significantly higher on control plants and plants treated with chlorantraniliprole than plants treated with thiacloprid. Ten out of the 21 insects in the thiacloprid treatment died after 24 h. No predator deaths were recorded on control or on chlorantraniliprole-treated plants at 24 h. No additional deaths were recorded at 72 h.

4. Discussion

Chlorantraniliprole did not cause significant mortality to *M. pygmaeus* (Fig. 1). Additional studies confirm the relative safety of chlorantraniliprole to insects other than Lepidoptera. For

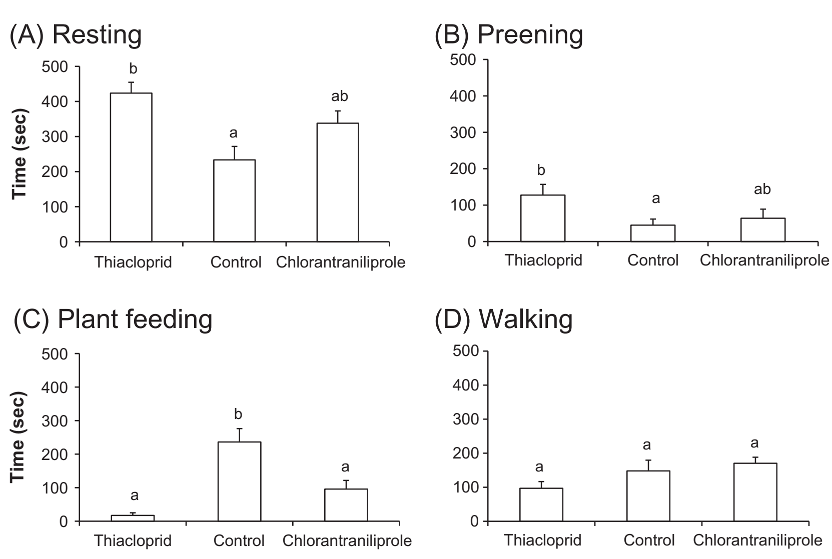

Fig. 2. Time in seconds allocated to resting, preening, plant feeding and walking (mean ± 1SE) by fifth-instar nymphs of *M. pygmaeus* on plants treated with either chlorantraniliprole, thiacloprid or distilled water (control). Duration of the observation period was 11 min. Different letters for the same behavior among treatments indicate statistically significant differences ($P < 0.05$, one-way ANOVA followed by the Tukey multiple comparison test).

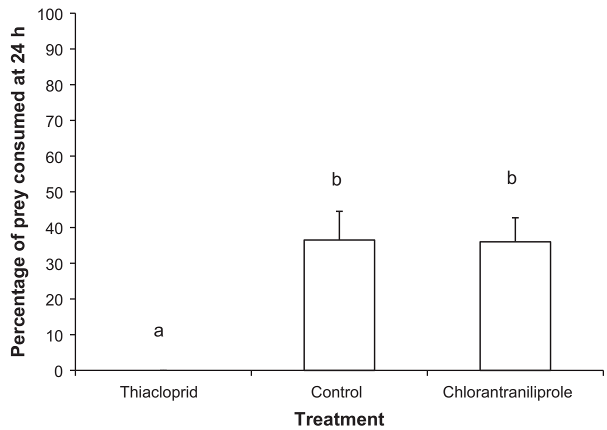

Fig. 3. Percentage of *E. kuehniella* eggs consumed by fifth-instar *M. pygmaeus* nymphs (mean ± 1SE) after 24 h on plants treated with either chlorantraniliprole, thiacloprid or distilled water (control). Different letters indicate statistically significant differences ($P < 0.05$, one-way ANOVA followed by the Tukey multiple comparison test).

example, chlorantraniliprole did not cause significant mortality to either bumble bees (*Bombus impatiens*) (Gradish et al., 2010), the rice water weevil (*Lissorhoptrus oryzophilus*), (Lanka et al., 2013), and a suite of parasitoids and ground beneficial arthropods (Brugger et al., 2010; Campos et al., 2011; Larson et al., 2012).

Emamectin benzoate, spinosad, and indoxacarb caused similar mortality to *M. pygmaeus* (Fig. 1). Emamectin benzoate is classified as harmless, as it caused less than 25% mortality, while indoxacarb and spinosad caused slightly higher than 25% mortality and they are classified as slightly harmful (Sterk et al., 1999). Studies in Spain showed that emamectin benzoate is harmless to *M. pygmaeus* (Lopez et al., 2011). However, emamectin benzoate induced high mortality to *Orius insidiosus*, another Hemipteran predator, (Studebaker and Kring, 2003; Biondi et al., 2012b), and therefore its safety seems to be species dependent. Arnó and Gabara (2011) reported benign effects of spinosad to *M. pygmaeus*, as it caused lower than 10% mortality to nymphs and adults following exposure to sprayed tomato leaves. Differences in *M. pygmaeus* mortality between the current and previous studies maybe due to differential susceptibility of the strains used, or differences in methodology, as our study applied a worst-case scenario by subjecting insects to three routes of pesticide exposure: Direct, residual and oral. In an extensive literature review, Biondi et al. (2012a) showed that spinosad effects on Hemiptera and other insect orders varied depending on species and rate used. Overall, Biondi et al. (2012a) recommended caution in the use of spinosad because of its negative side effects to many non-target species, including hymenopteran parasitoids.

Arnó and Gabarra (2011) also showed that indoxacarb caused 30% mortality to nymphs of *M. pygmaeus.* However, Galvan et al. (2006) reported that indoxacarb caused close to 100% mortality to 3rd instars of the lady beetle *Harmonia axyridis*. In contrast, Liu and Zhang (2012) reported that indoxacarb residues caused less than 20% mortality to adults of the hymenopteran parasitoids *Trichogramma pretiosum* and *T. brassicae*, indicating a species-specific action of the pesticide.

Interestingly, metaflumizone, a voltage dependent sodium channel blocker, that shares the same mode of action as indoxacarb (Table 1), caused close to 80% mortality to *M. pygmaeus* nymphs and it is classified as harmful (Fig. 1). Similarly, Biondi et al. (2012b) reported that the insecticide caused higher than 80% mortality to another Hemipteran predator, the mirid *Orius laevigatus*. The higher toxicity of metaflumizone compared to indoxacarb, despite their common mode of action, may be due to their different chemical structure (Table 1).

Copper hydroxide caused close to 60% mortality to *M. pygmaeus* nymphs and it is characterized as moderately harmful. Previous work has shown no important effects of copper hydroxide on the parasitoid *Tamarixia radiata* (Hall and Nguyen, 2010). However, Michaud and Grant (2003) have demonstrated negative effects of copper sulphate formulations on nematodes, parasitoids, and ladybeetles.

Thiacloprid, caused 100% mortality to *M. pygmaeus* nymphs (Fig. 1). The result was not surprising since thiacloprid is recommended against Hemipteran pests, such as whiteflies (Table 1). Direct contact of thiacloprid with *M. pygmaeus* seems to be an important source of toxicity, as predators exposed to the pesticide through residues and food in the behavioral assay suffered around 50% mortality (see Results). Thiacloprid is also known for causing high mortality to other beneficial species, as Bastos et al. (2006) reported a 50% reduction in adult emergence of the hymenopteran parasitoid *T. pretiosum*. However, thiacloprid has been reported to be relatively safe to larvae of the dipteran hover fly *Episyrphus balteatus* (Moens et al., 2011) and to adults of the predatory mite *Neoseiulus fallacis* (Bostanian et al., 2010).

In addition to lethal effects, we evaluated the sublethal behavioral effects of chlorantraniliprole and thiacloprid, two pesticides with distinct modes of action (Table 1), that caused the lowest and highest mortality, respectively (Fig. 1). Because thiacloprid caused 100% mortality in the toxicity assay, we modified our approach by using unsprayed *M. pygmaeus* exposed only to two routes of pesticide exposure: residual via treated leaf surfaces and via treated food. The two routes of pesticide exposure simulated a scenario where the predator escaped direct contact with spray droplets and was later exposed to the pesticide via walking on treated surfaces and consuming treated egg prey or plant material. Insects on plants treated with thiacloprid spent significantly more time resting and preening, and significantly less time feeding from the plant than insects on control plants (Fig. 2). Insects on plants treated with chlorantraniliprole spent significantly less time feeding from the plant than insects on control plants. The behavioral effects of thiacloprid were stronger than those for chlorantraniliprole, probably because of its specificity against Hemipteran insects (Table 1).

There were no significant differences in walking time of *M. pygmaeus* between treatments (Fig. 2). Previous studies have shown increased or decreased mobility for insects after their exposure to organophosphates (Alix et al., 2001; Hoy and Dahlsten, 1984; Thornham et al., 2008) and pyrethrins (Cox and Wilson, 1984; Thornham et al., 2008) – see Desneux et al. (2007) for a review of effects of pesticides on insect mobility. More recent studies, have shown that effects of certain insecticides on insect mobility are dose dependent, as bees exposed to lower doses of imidacloprid at 1.25 ng per insect exhibited increased mobility, whereas exposure to higher doses ranging from 2.5 to 20 ng per insect reduced mobility (Lambin et al., 2001). Tran et al. (2004) found that *Neochrysocharis formosa* parasitoids exposed to imidacloprid spent less time walking on leaves and feeding from hosts and more time resting than untreated parasitoids. In contrast, chlorantraniliprole was not found to influence the mobility of the earwig *Doru luteipes*, an important natural enemy in maize fields of South America (Campos et al., 2011).

Thiacloprid affected the foraging behavior and predation rate of *M. pygmaeus* (Figs. 2 and 3). Predators on plants treated with thiacloprid spent less time feeding from the plant than predators on control plants (Fig. 2). In addition, predation rate at 24 h was significantly lower (zero) on plants treated with thiacloprid than on control plants (Fig. 3). Imidacloprid, another neonicotinoid, was also found to negatively influence the predation rate of the coccinelid *Serangium japonicum* (He et al., 2012). Insects on plants treated with chlorantraniliprole spent less time feeding from the plant than those on control plants, suggesting that the insecticide could affect their foraging behavior. However, there were no significant differences of predation rate at 24 h between insects on control or chlorantraniliprole-treated plants, suggesting that the effects of the insecticide are short-lived (Fig. 3). Similarly, Liu et al. (2012) found no significant effect of chlorantraniliprole on the searching behavior of the parasitoid *Anagrus nilaparvatae*, an important natural enemy of the rice plant hopper.

5. Conclusions

Through laboratory studies we have shown that pesticides used in tomato agro-ecosystems may have a variety of lethal and sublethal effects on *M. pygmaeus*, a key natural enemy of important pests. Acute toxicity studies suggested that chlorantraniliprole, emamectin benzoate, indoxacarb and spinosad are relatively compatible with *M. pygmaeus*, whereas more work needs to be carried out for copper hydroxide and metaflumizone. Thiacloprid is incompatible with the predator. Further sublethal tests for thiacloprid and chlorantraniliprole showed that thiacloprid altered the behavior of the predator and reduced its predation rate at 24 h, while chlorantraniliprole had milder behavioral effects. Future work on sublethal and residual effects of pesticides, as well as long-term laboratory and field studies will advance our understanding of the impact of pesticides on *M. pygmaeus* and other non-target organisms.

Acknowledgements

We would like to thank Mr Athanasios Grigoriou and Ms Efstathia Eleftheriou for their technical assistance during the experiments. This work was funded by a grant from the Cyprus University of Technology to M.C. Stavrinides.

References

Alix, A., Cortesero, A.M., Nenon, J.P., Anger, J.P., 2001. Selectivity assessment of chlorfenvinphos re-evaluated by including physiological and behavioral effects on an important beneficial insect. Environ. Toxicol. 20, 2530–2536.
Arnó, J., Gabarra, R., 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J. Pest Sci. 84, 513–520.
Bastos, C.S., de Almeida, R.P., Suinaga, F.A., 2006. Selectivity of pesticides used on cotton (*Gossypium hirsutum*) to *Trichogramma pretiosum* reared on two laboratory-reared hosts. Pest Manage. Sci. 62, 91–98.
Bernstein, C., Kacelnik, A., Krebs, J.R., 1988. Individual decisions and the distribution of predators in a patchy environment. J. Anim. Ecol. 57, 1007–1026.
Biondi, A., Mommaerts, V., Smagghe, G., Viñuela, E., Zappalà, L., Desneux, N., 2012a. The non-target impact of spinosyns on beneficial arthropods. Pest Manage. Sci. 68, 1523–1536.
Biondi, A., Desneux, N., Siscaro, G., Zappalà, L., 2012b. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87, 803–812.
Bostanian, N.J., Hardman, J.M., Thistlewood, H.A., Racette, G., 2010. Effects of six selected orchard insecticides on *Neoseiulus fallacis* (Acari: Phytoseiidae) in the laboratory. Pest Manage. Sci. 66, 1263–1267.
Brugger, K.E., Cole, P.G., Newman, I.C., Parker, N., Scholz, B., Suvagia, P., Walker, G., Hammond, T.G., 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Manage. Sci. 66, 1075–1081.
Campos, M.R., Picanço, M.C., Martins, J.C., Tomaz, A.C., Guedes, R.N.C., 2011. Insecticide selectivity and behavioral response of the earwig *Doru luteipes*. Crop Prot. 30, 1535–1540.
Candolfi, MP, Barrett, KL, Campbell, P, Forster, R, Grandy, N, Huet, MC, Lewis, G, Oomen, PA, Schmuck, R, Vogt, H, 2001. Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. In: SETAC/ESCORT2 Workshop report. 21–23 March 2000, Wageningen.
Cordova, D., Benner, E.A., Sacher, M.D., Rauh, J.J., Sopa, J.S., Lahm, G.P., Selby, T.P., Stevenson, T.M., Flexner, L., Gutteridge, S., Rhoades, D.F., Wu, L., Smith, R.M., Tao, Y., 2006. Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic. Biochem. Physiol. 84, 196–214.
Cox, R.L., Wilson, W.T., 1984. Effects of permethrin on the behavior of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Environ. Entomol. 13, 375–378.
Croft, B.A., 1990. Arthropod Biological Control Agents and Pesticides. Wiley and Sons, New York.
Delpuech, J.-M., Dupont, C., Allemand, R., 2012. Effects of deltamethrin on the specific discrimination of sex pheromones in two sympatric *Trichogramma species*. Ecotoxicol. Environ. Saf. 84, 32–38.
Desneux, N., Wajnberg, E., Fauvergue, X., Privet, S., Kaiser, L., 2004. Sublethal effects of a neurotoxic insecticide on the oviposition behavior and the patch-time allocation in two aphid parasitoids, *Diaeretiella rapae* and *Aphidius matricariae*. Entomol. Exp. Appl. 112, 227–235.
Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.
Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C.A., González-Cabrera, J., Catalán Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, history of invasion and prospects for biological control. J. Pest Sci. 83, 197–215.
Desneux, N., Luna, M.G., Guillemaud, T., Urbaneja, A., 2011. The invasive South American tomato pinworm, Tuta absoluta, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J. Pest Sci. 84, 403–408.
Dixon, A.F.G., 2000. Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control. Cambridge University Press, Cambridge, UK.
Elbert, A., Haas, M., Springer, B., Thielert, W., Nauen, R., 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manage. Sci. 64, 1099–1105.
Galvan, T.L., Koch, R.L., Hutchinson, D.W., 2006. Toxicity of indoxacarb and spinosad to the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), via three routes of exposure. Pest Manage. Sci. 62, 797–804.
Gardiner, M.M., Fiedler, A.K., Costamagna, A.C., Landis, D.A., 2011. Integrating conservation biological control into IPM systems. In: Radcliffe, E.B., Hutchison, W.D. (Eds.), Integrated Pest Management. Cambridge University Press, Cambridge, pp. 151–162.
Gradish, A., Scott-Dupree, C., Shipp, L., Harris, R., Ferguson, G., 2010. Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). Pest Manage. Sci. 66, 142–146.
Hall, D.G., Nguyen, R., 2010. Toxicity of pesticides to *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid. BioControl 55, 601–611.
Hassan, S.A., Halsall, N., Gray, A.P., Kuehner, C., Moll, M., Bakker, F.M., Roembke, J., Yousef, A., Nasr, F., Abdelgader, H., 2000. Laboratory method to evaluate the side effects of plant protection products on Trichogramma cacoeciae Marchal (Hym., Trichogrammatidae). In: Candolfi, M.P., Blümel, S., Forster, R., Bakker, F.M., Grimm, C., Hassan, S.A., Heimbach, U., Mead-Briggs, M.A., Reber, B., Schmuck, R., Voght, H. (Eds.), Guidelines to Evaluate Side Effects of Plant Protection Products to non-Target Arthropods. Gent, IOBC/WPRS, pp. 107–119.
He, Y., Zhao, J., Zheng, Y., Desneux, N., Wu, K., 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology 21, 1291–1300.
Hoy, J.B., Dahlsten, D.L., 1984. Effects of malathion and Staley's bait on the behavior and survival of parasitic hymenoptera. Environ. Entomol. 13, 1483–1486.
Lambin, M., Armengaud, C., Raymond, S., Gauthier, M., 2001. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch. Insect Biochem. 48, 129–134.
Lanka, S.K., Ottea, J.A., Davis, J.A., Hernandez, A.B., Stout, M.J., 2013. Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in rice. Pest Manage. Sci. 69, 250–256.
Larson, J.L., Redmond, C.T., Potter, D.A., 2012. Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass. Pest Manage. Sci. 68, 740–748.
Liu, T.-X., Zhang, Y., 2012. Side effects of two reduced-risk insecticides, indoxacarb and spinosad, on two species of *Trichogramma* (Hymenoptera: Trichogrammatidae) on cabbage. Ecotoxicology 21, 2254–2263.
Liu, F., Zhang, X., Gui, Q.Q., Xu, Q.-J., 2012. Sublethal effects of four insecticides on *Anagrus nilaparvatae* (Hymenoptera: Mymaridae), an important egg parasitoid of the rice planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). Crop Prot. 37, 13–19.
Lopez, J.A., Amor, F., Bengochea, P., Medina, P., Budia, F., Viñuela, E., 2011. Short communication. Toxicity of emamectin benzoate to adults of *Nesidiocoris tenuis* Reuter, *Macrolophus pygmaeus* (Rambur) and *Diglyphus isaea* Walker on tomato plants. Semi-field Stud. Span. J. Agric. Res. 9, 617–622.
Lu, Y., Wu, K., Jiang, Y., Guo, Y., Desneux, N., 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487, 362–365.
Lykouressis, D., Giatropoulos, A., Perdikis, D., Favas, C., 2008. Assessing the suitability of noncultivated plants and associated insect prey as food sources for the omnivorous predator *Macrolophus pygmaeus* (Hemiptera: Miridae). Biol. Control 44, 142–148.
Martinou, A.F., Wright, D.J., 2009. The predation consequence of continuous breeding vs starting a new colony of a polyphagous insect predator. Phytoparasitica 37, 27–33.
Martinou, A.F., Milonas, P.G., Wright, D.J., 2009. Patch residence decisions made by *Aphidius colemani* in the presence of a facultative predator. Biol. Control 49, 234–238.
Michaud, J.P., Grant, A.K., 2003. Sub-lethal effects of a copper sulfate fungicide on development and reproduction in three coccinellid species. J. Insect Sci. 3, 1–6.

Moens, J., Clercq, P.D., Tirry, L., 2011. Side effects of pesticides on the larvae of the hoverfly *Episyrphus balteatus* in the laboratory. Phytoparasitica 39, 1–9.

Nakashima, Y., Hirose, Y., 2003. Sex differences in foraging behavior and oviposition site preference in an insect predator *Orius sauteri*. Entomol. Exp. Appl. 106, 79–86.

Pekár, S., 2012. Spiders (Araneae) in the pesticide world: an ecotoxicological review. Pest Manage. Sci. 68, 1438–1446.

Perdikis, D., Fantinou, A., Lykouressis, D., 2011. Enhancing pest control in annual crops by conservation of predatory Heteroptera. Biol. Control 59, 13–21.

R Development Core Team, 2006. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. http://www.R-project.org.

Stark, J.D., Vargas, R., Banks, J.E., 2007. Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J. Econ. Entomol. 100, 1027–1032.

Stavrinides, M.C., Mills, N.J., 2009. Demographic effects of pesticides on biological control of Pacific spider mite (*Tetranychus pacificus*) by the western predatory mite (*Galendromus occidentalis*). Biol. Control 48, 267–273.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blümel, S., Bogenschütz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Rovesti, L., Samsoe-Peterson, L., Sauphanor, B., Schaub, L., Stäubli, A., Tuset, J.J., Vainio, A., de Veire, M.V., Viggiani, G., Viñuela, E., Vogt, H., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". BioControl 44, 99–117.

Studebaker, G., Kring, T., 2003. Effects of insecticides on *Orius insidiosus* (Hemipreta: Anthocoridae), measured by field, greenhouse and Petri dish bioassays. Fla. Entomol. 86, 178–185.

Thornham, D.G., Blackwell, A., Evans, K.A., Wakefield, M., Walters, K.F.A., 2008. Locomotory behavior of the seven-spotted ladybird, *Coccinella septempunctata*, in response to five commonly used insecticides. Annu. Appl. Biol. 152, 349–359.

Tran, D.H., Takagi, M., Takasu, K., 2004. Effects of selective insecticides on host searching and oviposition behavior of *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a larval parasitoid of the American serpentine leafminer. Appl. Entomol. Zool. 39, 435–441.

Urbaneja, A., Montón, H., Molla, O., 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292–296.

Urbaneja, A., González-Cabrera, J., Arnó, J., Gabarra, R., 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manage. Sci. 68, 1215–1222.

van Laerhoven, S.L., Gillespie, D.R., McGregor, R.R., 2000. Leaf damage and prey type determine search effort in *Orius tristicolor*. Entomol. Exp. Appl. 97, 167–174.

van Laerhoven, S.L., Gillespie, D.R., Roitberg, B.D., 2006. Patch retention time in an omnivore, *Dicyphus hesperus* is dependent on both host plant and prey type. J. Insect Behav. 19, 613–621.

Wrinn, K.M., Evans, S.C., Rypstra, A., 2012. Predator cues and herbicide affect activity and emigration in agrobiont wolf spider. Chemosphere 87, 390–396.

Yano, E., Jiang, N., Hemerik, L., Mochizuki, M., Mitsunaga, T., Shimoda, T., 2005. Time allocation of *Orius sauteri* in attacking *Thrips palmi* on an eggplant leaf. Entomol. Exp. Appl. 117, 177–184.

Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. Ecol. Econ. 64, 253–260.