

Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system

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Abstract *Coccinella undecimpunctata* L. is a euryphagous predator established in Azores that offers interesting potential as a control agent in the context of integrated pest management (IPM). However, to further develop IPM against aphids, it is important to evaluate the effects that insecticides might have on voracity. Thus, we proposed to evaluate the effects of pirimicarb and pymetrozine on the voracity of 4th instar larvae and adults of *C. undecimpunctata*, under distinct scenarios of exposure to chemicals within a prey/plant system. Voracity of *C. undecimpunctata* was not significantly affected by pirimicarb or pymetrozine when treatments were directly sprayed on the predator; however, when insecticides were sprayed on the prey/plant system, the predator's voracity was significantly increased. Results suggest that *C. undecimpunctata* does not detect the insecticide on the aphids and indicate that the increase in voracity may be due to a decrease in the mobility of insecticide-treated aphids, since their capture should be easier than highly mobile non-treated prey. The consequences of such increase in the voracity for IPM programs are discussed.

Keywords Coccinellidae · IPM · Insecticide side-effects · Pirimicarb · Pymetrozine

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Introduction

Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) is a euryphagous predator that feeds especially on aphids (Raimundo and Alves 1986; Hodek and Honěk 1996). Given its voracity toward these pests, *C. undecimpunctata* offers interesting potential as a control agent in the context of Integrated Pest Management (IPM) (ElHag 1992; Zaki et al. 1999; Moura et al. 2006; Cabral et al. 2006, 2008). The success of IPM programs depends, in part, on the optimal use of selective insecticides that are less harmful to natural enemies (Tillman and Mulrooney 2000; Stark et al. 2007), which requires knowledge of their side-effects on the biological and behavioural traits of these organisms (Tillman and Mulrooney 2000; Sechser et al. 2003; Youn et al. 2003; Liu and Stansly 2004; Bozski 2006; Desneux et al. 2007; Stark et al. 2007). Some studies have been done to assess the susceptibility of *C. undecimpunctata* to different insecticides but all, in some way, adversely affected this species (Salman and Abd-el-Raof 1979; Lowery and Isman 1995; Schroeder et al. 1996; Omar et al. 2002). Recent studies showed that, in general, pirimicarb and pymetrozine had no adverse effects on the biological traits (i.e. developmental time, fecundity, fertility, percentage of egg hatch) of immature or adult stages of *C. undecimpunctata* when sprayed on the insects, which makes these chemicals potentially suitable to use in combination with *C. undecimpunctata* for integrated control of sucking pests (Cabral et al. 2008).

However, to further develop IPM against aphids, it is important to evaluate the effects that these insecticides might have on *C. undecimpunctata* predatory capacity, since it is considered relevant to evaluate the predator's potential as a biological control agent (Holling 1959; ElHag and Zaitonn 1996; Omkar and Pervez 2004;

Tsaganou et al. 2004). Previous studies indicated that sublethal effects of insecticides may result in an immediate disruption of predatory behaviour and a potential reduction in the efficiency of coccinellids to locate and capture their prey, since chemicals may interfere with the feeding behaviour by repellent, antifeedant or reduced olfactory capacity effects (Singh et al. 2001, 2004; Stark et al. 2004, 2007; Desneux et al. 2007). The behavioural responses may also alter the predator's search pattern (Thornham et al. 2007, 2008) by avoidance of treated surfaces or ingestion of treated prey, to minimize their contact with insecticides (Wiles and Jepson 1994; Singh et al. 2001, 2004). On the other hand, insecticides can indirectly induce modifications on the dynamic predator/prey, through changes in the state and behaviour of the aphid colony that will influence relative prey value and consequently the predator's active choice (Sih and Christensen 2001). In addition, reductions (or absence) in the mobility and of defensive responses by the aphids can influence the predator's choice, as shown by several authors (Eubanks and Denno 2000; Sih and Christensen 2001; Provost et al. 2006).

In the field, beneficial arthropods can be exposed to insecticides in several ways: by direct contact with spray droplets; by uptake of residues when contacting with contaminated plant surfaces; by ingestion of insecticide-contaminated prey, nectar or honeydew (i.e. uptake of insecticide-contaminated food sources) (Hurej and Dutcher 1994; Longley and Stark 1996; Obrycki and Kring 1998; Youn et al. 2003). Since it is known that the susceptibility of natural enemies to insecticides varies with the route of pesticide exposure (Longley and Jepson 1996a, b; Longley and Stark 1996; Banken and Stark 1998; Naranjo 2001; Grafton-Cardwell and Gu 2003), it is important to perform both topical and residual tests as they can provide valuable information about the expected and observed impacts of insecticides on natural enemies in the field (Tillman and Mulrooney 2000). On the other hand, in the field predator/prey interactions generally occur in structurally complex patches (i.e. plant architecture and surface features), which thereby influences the predator's foraging efficacy (Dixon 2000; Pettersson et al. 2005; Rutledge and O'Neil 2005). Thus, studies regarding insecticide effects on predator's voracity should also reflect such scenarios (i.e. the tri-trophic system predator/prey/plant), particularly when testing systemic insecticides where the presence of the plant allows prey contamination not only by contact, but also through the food source.

Some studies have addressed the susceptibility of immature and adult coccinellids to pirimicarb and pymetrozine, when directly sprayed on prey and/or predators (e.g. James 2002, 2003) but nothing is known about the side-effects of these chemicals on prey/predator interactions within tri-trophic systems. Hence, this study aimed to

evaluate the effects of these insecticides on the voracity of 4th instar larvae and adults of *C. undecimpunctata*, under two distinct scenarios of exposure to each chemical within a tri-trophic system: (1) exposure by direct contact: insecticide was sprayed directly on coccinellids and then predators were added to a prey/plant system (not treated with insecticide) and (2) exposure by contact with a insecticide-treated prey/plant system: non-treated predators were added to a prey/plant system previously sprayed with the insecticide.

Materials and methods

Insects

Adults of *C. undecimpunctata* were collected in Sta. Maria Island, Azores, Portugal, early in the summer (July 2006). Coccinellids were reared in net cages (50 × 50 × 50 cm), at 25 ± 1°C, 75 ± 5% RH and a photoperiod of 16L:8D, using fluorescent lamps (Sylvania Standard F36 W/133). The coccinellids were provided with an ad libitum mixed diet of different developmental stages of aphids [*Aphis fabae* (Scopoli) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae)] on *Vicia faba* plants, multiflower bee pollen, and a solution of honey diluted in water (30%) applied to cotton. Individuals (4th instar larvae and adults) produced in this rearing system were used for the experiments.

Insecticide experiments

The effects of pirimicarb (Pirimor G, WG [0.375 g (AI)/L], Syngenta; neurotoxic) and pymetrozine (Plenum, WP [0.2 g (AI)/L], Syngenta; antifeedant) on the predation efficiency of *C. undecimpunctata* (4th instar larvae and adult males and females) within a tri-trophic system were evaluated using *M. persicae* as prey and *Vicia faba* as host plant. Coccinellids were exposed to insecticides using two distinct scenarios: (1) direct contact by topical application of the insecticide on the coccinellids and (2) exposure of coccinellids to a previously insecticide-treated prey/plant system.

The insecticides were selected based on their current and potential use for the management of aphids (Jansen 2000; James 2002) and because they represent neuroactive insecticides from distinct chemical families, and thus with distinct modes of action. Pirimicarb acts upon contact, disrupting the neuronal communication through the inhibition of acetylcholinesterase, having a quick knockdown effect, accompanied by paralysis, tremor and insect death (Fukuto 1990). Pymetrozine acts systemically, interfering with neuroregulation or nerve-muscle interaction, which results in feeding inhibition, followed by delayed death through starvation. Physiologically, pymetrozine appears to act by preventing plant-sucking insects from inserting their

stylus into the plant tissue (Ausborn et al. 2005). In addition, these insecticides are considered to have relatively low toxicity to several beneficial insects and mites, such as Coccinellidae, Phytoseiidae, Chrysopidae and Aphelinidae (Sterk et al. 1999; Jansen 2000; James 2002; Desneux et al. 2004), being therefore recommended as suitable for IPM by the manufacturers.

For each insecticide, fresh solutions diluted with distilled water were prepared at the doses recommended by the manufacturer for the control of aphids (pirimicarb 375 g ai/ha; pymetrozine 200 g ai/ha). Solutions were sprayed on coccinellids or prey/plant system [6 ml of the aqueous suspension of the insecticide or distilled water (control treatment)] using a Potter Tower (Burkard, Rickmansworth, UK) at 2 kPa. This resulted in homogeneous spray coverage of $9.52 \pm 2.17 \mu\text{l}$ (mean \pm SD) of fluid per cm^2 , corresponding to an application rate of 1,000 l/ha.

All bioassays were performed at $25 \pm 1^\circ\text{C}$, $75 \pm 5\%$ RH and a photoperiod of 16L:8D using fluorescent lamps (Sylvania Standard F36 W/133).

Exposure of coccinellids to insecticides by direct contact

Prior to the insecticide or control treatments, larvae (12–24 h after moulting to the 4th instar) or adults (12–24 h after emergence) of *C. undecimpunctata* were placed into plastic Petri dish bottoms (150 \times 15 mm) and held for 30 min at 15°C to reduce the insects' mobility. Larvae and adults (males and females) were sprayed individually. After spraying, each individual was transferred to an untreated plastic box (24 cm in height, 7 cm in width and 12 cm in depth) containing a small *V. faba* plant (approximately 10 cm high growing in a pot with soil) and *M. persicae* (nymphs of the 4th instar and adults). The number of prey per predator corresponded to the minimum necessary to satiate during 24 h appropriate to the developmental stage of the predator, previously determined by Cabral et al. (2009): 130 aphids per 4th instar larva and 90 aphids per adult (male or female).

Predation efficiency of *C. undecimpunctata* was evaluated through the determination of the voracity following the model of Soares et al. (2003):

$$V_o = (A - a_{24}) \text{ ra}_{24}$$

where V_o = number of eaten aphids, A = number of aphids available at the start of the experiment, a_{24} = number of aphids alive after 24 h and ra_{24} = ratio of aphids alive after 24 h in the absence of predators.

The procedure was repeated at least 15 times per insecticide or control treatments for 4th instar, adult male and female of *C. undecimpunctata*.

Exposure of coccinellids to insecticides by contact with an insecticide-treated prey/plant system

Larvae (12–24 h after moulting to the 4th instar) or adults (12–24 h after emergence) of *C. undecimpunctata* were individually transferred to a plastic box (as above) with a small *V. faba* plant and *M. persicae* (nymphs of the 4th instar and adults) that had previously been sprayed with the insecticide or distilled water (control treatment), as described above. The prey/plant system was held for drying for 2 h, before adding the coccinellids into the system. The number of prey per predator corresponded to the minimum necessary to satiate during 24 h as before determined by Cabral et al. (2009): 130 aphids per 4th instar larva and 90 aphids per adult (male or female).

In this set of experiments, voracity of *C. undecimpunctata* was evaluated as:

$$V_o = A - a_{24}$$

where V_o = number of eaten aphids, A = number of aphids available, a_{24} = number of aphids not consumed (dead or alive) in the system after 24 h.

In this case, we had to adjust the voracity model by Soares et al. (2003) (where only the alive aphids are considered as not consumed), since most of the aphids were already dead due to the insecticide treatments when the predator was added to the prey/plant system.

The procedure was repeated at least 10 times per insecticide or control treatments for 4th instar larvae, adult male and female of *C. undecimpunctata*.

An additional test was performed using a similar tri-trophic set-up, but with dead frozen aphids instead of insecticide-treated dead aphids, to evaluate the impact of non-mobile prey (i.e. frozen aphids) on the voracity of the predator. To freeze the aphids, they were placed in a freezer for 30 min at -3°C , resulting in aphid death.

Statistical analysis

Voracity data of *C. undecimpunctata* 4th instar larvae and adults (females or males) were compared between the different insecticides and control treatments by one-way ANOVA. When ANOVA showed significant differences ($P < 0.05$) among data sets, paired comparisons of each mean were made using Fisher's protected LSD tests (Zar 1996). To reduce variance differences, data were transformed by $\log(x)$ (Zar 1996). All analyses were performed using SPSS v. 12.0 Windows (SPSS Inc. 2004).

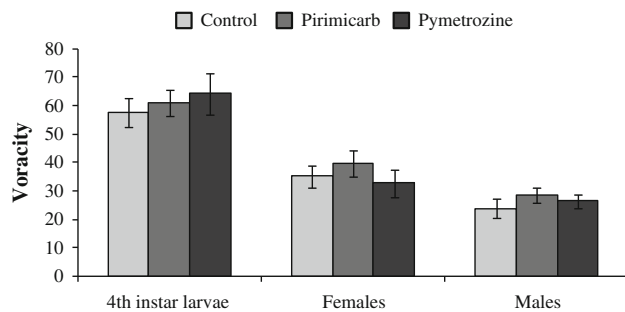


Fig. 1 Mean (\pm SE) voracity of *C. undecimpunctata* 4th instar larvae and adults (females or males) when treated with pirimicarb, pymetrozine or distilled water (control treatment), using *M. persicae* as prey

Results

Voracity of *C. undecimpunctata* was not significantly affected by pirimicarb or pymetrozine, when treatments were applied directly on the predator ($F_{(2,39)} = 0.503$, $P = 0.608$ for 4th instar larvae; $F_{(2,36)} = 0.915$, $P = 0.409$ for adult females; and $F_{(2,31)} = 0.964$, $P = 0.393$ for adult males) (Fig. 1). In general, regardless of the treatment, 4th instar larvae were significantly more voracious ($F_{(2,38)} = 20.3$, control; $F_{(2,38)} = 20.6$, pirimicarb; and $F_{(2,38)} = 28.9$, pymetrozine; all $P < 0.0001$), consuming an average of 60 aphids (Fig. 1).

However, when the treatment was carried on the prey/plant system, the voracity of *C. undecimpunctata* was significantly higher than in control treatments in insecticide-treated systems ($F_{(3,41)} = 2.906$, $P = 0.04$ for 4th instar larvae; $F_{(3,40)} = 5.92$, $P = 0.02$ for adult females; and $F_{(3,39)} = 5.57$, $P = 0.03$ for adult males) (Fig. 2).

The highest voracity, regardless of the developmental stage or sex of *C. undecimpunctata*, was observed in prey/plant systems treated with pirimicarb, being in all tests significantly different from the control (distilled water) and from pymetrozine-treated system when males were tested (Fig. 2).

Also, the voracity of *C. undecimpunctata* in the prey/plant system with frozen aphids was higher than the control, regardless of the tested developmental stage or sex, and did not differ significantly from the pirimicarb-treated prey/plant system (Fig. 2).

In these tests, the pattern of a significantly higher voracity of 4th instar larvae is also present ($F_{(2,26)} = 34.75$, control; $F_{(2,27)} = 23.51$, pirimicarb; $F_{(2,27)} = 30.04$, pymetrozine; and $F_{(2,37)} = 27.82$, frozen; all $P < 0.0001$) (Fig. 2).

Discussion

To our knowledge, there are very few studies to report the effects of insecticides on the voracity of coccinellid

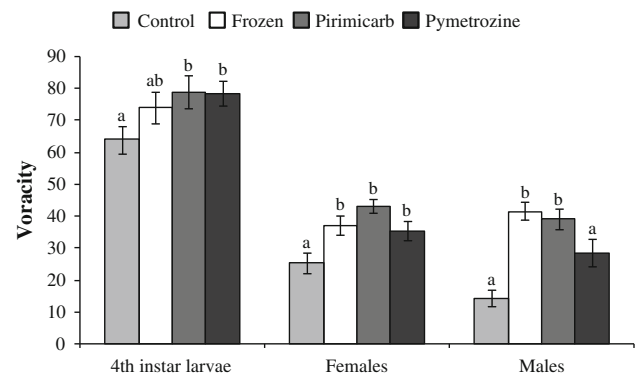


Fig. 2 Mean (\pm SE) voracity of *C. undecimpunctata* 4th instar larvae and adults (females or males) using *M. persicae* as prey within the following prey/plant systems: prey/plant systems treated with pirimicarb, pymetrozine or distilled water (control treatment); and prey/plant system not treated with insecticides but with prey previously killed by freezing. Bars, for a group, with different letters are significantly different at $P < 0.05$ (LSD test)

predator when foraging in a prey/plant system. The voracity of *C. undecimpunctata* was not significantly affected by pirimicarb or pymetrozine after the predator has been exposed to the insecticides by direct contact. Moura et al. (2006) obtained a similar result when testing pirimicarb on *C. undecimpunctata* feeding on *A. fabae*. Similarly, Roger et al. (1994, 1995) observed that three other insecticides (cypermethrin, carbaryl and malathion) did not significantly affect the mean daily aphid consumption by adults of *C. maculata lengi*. Garcia (1979) observed that 4th instar larvae of *C. sulphurea* and *Semidalia undecimnotata* (Coleoptera: Coccinellidae) reduced the number of prey consumed after direct exposure to an identical dose of pirimicarb. Topical exposure to other pesticides such as benomyl and azadirachtin on *C. maculata lengi* (Roger et al. 1994, 1995) and lambda-cyhalothrin on *Harmonia axyridis* (Provost et al. 2005) significantly decreased the rate of aphid consumption.

Surprisingly, voracity of *C. undecimpunctata* was higher when predators were added to a pirimicarb or pymetrozine previously sprayed prey/plant system. The astonishment of such results relies on the fact that the majority of studies indicate reductions in the consumption of insecticide-treated prey [e.g. lambda-cyhalothrin, cypermethrin (Thornham et al. 2007) and dimethoate on *C. septempunctata* (Singh et al. 2004) and endosulfan on *H. conformis* (Qi et al. 2001)] and few had shown no changes on the consumption rates of coccinellids on insecticide-treated prey, such as pirimicarb, dimethoate and chlorpyrifos on *C. septempunctata* (Thornham et al. 2007) and azadirachtin on *H. conformis* (Qi et al. 2001), but no other study have shown an increase in the consumption of insecticide-treated prey.

Further than suggesting that *C. undecimpunctata* is not be able to detect the insecticides on aphids, the increase in consumption may be due to decrease in prey mobility (on pymetrozine-treated aphids) or total immobility (on pirimicarb-treated aphids), since their capture should be easier than the highly mobile non-treated prey (control group). The consumption of frozen aphids supports this hypothesis given that in this treatment, the number of prey eaten was significantly higher than the observed in the control, and it was similar to pirimicarb-treated prey (where all prey were dead, therefore immobile) regardless the predators' developmental stage or sex. According to Sih and Christensen (2001), capture success will depend on prey and predator mobility, and the susceptibility to be consumed upon capture will depend on the defensive prey behaviours; prey having better escape responses are less prone to be consumed. Thus, prey mobility and their defensive behaviours can influence prey selection and capture (Eubanks and Denno 2000; Provost et al. 2006). Aphids are known to exhibit a wide range of behavioural (e.g. kick, attack with frontal horns, walk away and drop from the plant), chemical and morphological defences (Ferran and Dixon 1993; Dixon 2000). Moreover, all the above-mentioned studies were done in simple arenas (e.g. Petri dishes) without the plant, but the morphological complexity of plants can provide a physical refuge to the prey and/or interfere with the foraging activities of a natural enemy, reducing the predation efficacy (Clark and Messina 1998; Dixon 2000; Guershon and Gerling 2006; Legrand and Barbosa 2003; Rutledge and O'Neil 2005).

Several studies have demonstrated that the ingestion of insecticide-treated prey can have negative side-effects on coccinellids, such as reduced longevity, fecundity, oviposition period and length of pre-oviposition period (Olszak et al. 1994; Kalushkov 1999; Olszak 1999; Grafton-Cardwell and Gu 2003; Liu and Stansly 2004; Angeli et al. 2005; Wang et al. 2005; Kim et al. 2006). Since nothing is known about such effects when *C. undecimpunctata* feeds on pirimicarb- or pymetrozine-treated prey, further studies are required to determine whether these insecticides could be safely used in IPM programs. Several studies have demonstrated that beneficial insects, such as coccinellids and parasitoids, may be able to detect and respond to insecticides in their environment by changing their searching behaviour to avoid treated areas (Perera 1982; Bos and Masson 1983; Hoy and Dahlsten 1984; Longley and Jepson 1996a, b; Desneux et al. 2005; Thornham et al. 2007). However, our results suggest that *C. undecimpunctata* may be not able to detect the tested insecticides, because predators consumed more treated than untreated prey. The absence of a suitable capacity to reject treated prey may have a negative impact in the predators' efficacy in IPM programs, given that the predator would become

satiated before consuming the aphids that are alive (i.e. non-treated ones). Nevertheless, our results were from no-choice experiments, thus further studies are required to understand foraging behaviour and preferences of *C. undecimpunctata*, when insecticide-treated and non-treated prey are simultaneously given to the predator.

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