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SUBLETHAL EFFECTS OF THE INGESTION OF IMIDACLOPRID AND DELTAMETHRIN BY *CHRYSOPERLA AGILIS* (NEUROPTERA: CHRYSOPIDAE)

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ABSTRACT

The sublethal effects of two commonly used pesticides, Deltamethrin (Pyrethroid) and Imidacloprid (Chloronicotinyl) on the auxiliary insect *Chrysoperla agilis* (Neuroptera: Chrysopidae), were assessed. We followed the development and survival of immature stages fed on contaminated food, and evaluated the survival and sex-ratio of the emerged adults. For the ingestion essays, insecticides were sprayed on the larvae food (*Ephestia kuehniella* eggs) at doses recommended by the manufacturers for the control of aphids and whiteflies, using third instar larvae (L3) of *C. agilis* as predator. Deltamethrin treatment resulted in a high mortality rate amongst females, leading to a significant male biased sex-ratio when compared to Imidacloprid and control treatments, with 82.1% of male when adult stages were sexed. The weight gain of L3 was significantly lower after treatment with Deltamethrin compared both to control and Imidacloprid treatments. Contrarily, pupae weight gain was lower in Imidacloprid treated group, the only one differing significantly from the control. This shift in the weight gain (larvae vs. pupae) indicates a recovery trend in the Deltamethrin treated group that results in the absence of significant differences in the weight of the adults.

RESUMO

Foram avaliados os efeitos letais de dois pesticidas comummente usados no insecto auxiliar *Chrysoperla agilis* (Neuroptera: Chrysopidae), Deltamethrin (Pyrethroid) e Imidacloprid (Chloronicotinyl). Seguiu-se o desenvolvimento e sobrevivência dos estádios imaturos alimentados com comida contaminada e avaliou-se a sobrevivência e rácio sexual dos adultos que emergiram. Para os ensaios de ingestão, os insecticidas foram pulverizados no alimento das larvas (ovos de *Ephestia kuehniella*) com as doses recomendadas pelos fabricantes para controlo de afídeos e mosca-branca, usando como predador larvas de *C. agilis* no terceiro instar (L3). O tratamento com Deltamethrin resultou em elevada taxa de mortalidade entre as fêmeas, levando a um rácio sexual enviezado para macho quando comparado com o tratamento com Imidacloprid e com o controlo, com 82,1% de machos quando os estádios adultos foram inspeccionados. O ganho de peso das L3 foi significativamente mais baixo depois do tratamento com Deltamethrin, em comparação com o controlo e com o tratamento com Imidacloprid. Pelo contrário, o ganho em peso das pupas foi mais baixo no grupo tratado com Imidacloprid, o único significativamente diferente do controlo. Esta mudança no ganho de peso (larvas *vs*. pupas) indicia uma tendência de recuperação no grupo tratado com Deltamethrin que resulta na ausência de diferenças significativas no peso dos adultos.

INTRODUCTION

The agricultural model of the Azores is characterized by cycles of agricultural specialization resulting in the depletion of arable land and the emergence of pests, among other causes (Calado *et al*., 2011). Nowadays, while insecticide use is still one of the main pest management tools available in most agricultural settings (Cooper & Dobson, 2007; Edwards-Jones, 2008), there are increasing efforts to put forward integrated pest management (IPM) methods (Kogan, 1998) and a change in attitudes regarding the use of these compounds, as producers and consumers raise concerns about their safety (Matsumura, 2004; Matthews, 2008). This willingness to incorporate more sustainable practices has fueled studies regarding insecticide selectivity and impact on non-target species and their negative impact have been extensively recognised (Stark & Banks, 2003; Desneux *et al*., 2007; Stark *et al*., 2007). Since the outset of IPM programs, broad-spectrum pesticides have raised concern among practitioners as they are limited in their compatibility with most biological control agents (Stark *et al*., 2007; Jepson, 2007; Schneider *et al*., 2009). Moreover, the effect of pesticides on non-targeted organisms has traditionally relied on acute lethal effect such as LD50 or LC50 (lethal dose or concentration that kills 50% of a population) (Stark *et al*., 2007; Cordeiro *et al*., 2010). While it's a fast and cheap method to evaluate and compare the effect of several toxicants on arthropod species, sublethal responses to insecticides are even more important than mortality for both pest and non-target organisms (Stark & Banks, 2003; Desneux *et al*., 2007; Guedes *et al*., 2009). Target pest species and their natural enemies are predominantly exposed to sublethal concentrations in agricultural settings (Cordeiro *et al*.; 2010) and therefore the assessment of their effects allow a better understanding of the long-term impact on the ecosystem in place (Forbes & Calow, 1999; Stark & Banks, 2003; Stark, 2005).

Many physiological parameters are affected by pesticides, and contact or ingestion of sublethal doses may result in reduced fecundity, changes in fertility rate and oviposition behavior, shortened life span and developmental times, offspring mutation and weight loss (Schneider *et al*., 2009). As described by Stark *et al.* (2007), demographic parameters estimation is an essential approach for ecological predictions of population growth when assessing the effects of pesticides in IPM programs.

Lacewings are polyphagous predators commonly found in agricultural systems around the world and their use in biological control is now widespread (Albuquerque *et al*., 1994; Senior & McEwen, 2001). *Chrysoperla agilis* (Henry *et al*., 2003) occurs naturally in the Azores islands (Ventura *et al*., 2005) and its potential as a biocontrol agent for several agricultural pests such as aphids and scale insects has been described (Mendes & Ventura 2010), turning it into a useful tool on integrated pest management programs (IPM) of several crop pests (Mendes & Ventura, 2010).

Few studies have evaluated the effect of insecticides on life history traits and population parameters of natural enemies of pests (Stark & Banks, 2003; Desneux *et al*., 2007; Stark *et al*., 2007). Desneux *et al*., (2007) published a review of researches about sublethal effects of pesticides on beneficial arthropods over the past 15 years, emphasizing the importance of taking those effects into account when evaluating an integrated pest management program. When characterizing beneficial organisms on their pesticide tolerance, it would be expensive in time and energy to investigate each species with every pesticide. In this regard, there is an agreement in the IOBC WPRS Working Group "Pesticides and Beneficial Organisms" in which a role model species is chosen to be relevant to the crop on which the pesticide under study is to be used (Bozsik, 2009). For the family Chrysopidae, *C. carnea* has been selected as a general predator (Hassan *et al*., 1985). Table 1 presents recent work done with pesticides on this species group. Only two species stand out when it comes to IPM programs, the neotropical *Chrysoperla externa* and the holarctic *Chrysoperla carnea*. However, when it comes to *C. carnea*, its holarctic distribution is no longer valid since the discovery of a complex of sibling species within this *taxon* (Thierry *et al*., 1992, 1994). In this context *C. agilis* has been described as a new species (Henry *et al*., 2003), being a part of the European *carnea*-complex. Thus, the results concerning the pesticide compatibility obtained for *C. carnea sensu lato*, cannot be applied anywhere since we may be dealing with different species, instead of different populations. This is also a problem when it comes to mass production. In consequence, lacewing species such as *C. agilis* have been rarely examined and the application of the toxicological values obtained for *C. carnea s. l.*, were hardly verified.

Chrysoperla agilis, as well as most species of lacewings, is entomophagous in the larval stage and primarily feed on nectar and pollen in the adult stage (Stelzl, 1991; Bozsik, 1992; Morgado *et al*., 2014). The objective of this study was to assess the sub-lethal effects of two commonly used pesticides in Azores, Imidacloprid and Deltamethrin, on the developmental parameters of the native *C. agilis* (Neuroptera: Chrysopidae), in the presence of contaminated food in the laboratory.

MATERIAL AND METHODS

Insects' rearing

The adults of *C. agilis* used in this study were collected in citrus orchards of S. Miguel Island (Azores), and reared in a climate chamber at 25 ± 0.5 °C temperature, 75 ± 5% RH, a photoperiod of 16:8 (L:D) h, and fed on *Myzus persicae* and *Ephestia (Anagasta) kuehniella* eggs.

Food sources for the essays

Ephestia kuehniella eggs were used to feed the larvae of *C. agilis* and obtained from a mass rear production of the Biology department, University of the Azores (Tavares, 1984). An artificial mixture of water: honey: yeast extract: commercial pollen (1:1:1:1), was used to feed the adults. Both food sources were kept refrigerated (c.a. ≈ 6 °C) during the essays.

Experimental unit

For each treatment, 30 *Chrysoperla agilis* larvae, originated from the same cohort (eggs laid within ≤ 12 h), were put inside 7 ml individual glass vials with a strip of paper as shelter and a stopper of hydrophilic cotton. Vials were placed into an acrylic box containing a salt solution to provide humidity (≈ 75% RH), and kept inside a Sanyo® chamber at 25 ± 0.5 °C temperature, and a photoperiod of 16:8 (L:D) h. The individuals were checked daily for the presence of an exuvia to determine the developmental stage and fed *ad libitum* every other day, with *E. kuehniella* eggs. Newly emerged third instar larvae (L_3) were then starved for 24 h in a glass vial as described below, and then weighted

TABLE 1. Summary of the literature published from 2002-2013 regarding the sublethal effects of pyrethroids on Chrysopidae predators. B, Behaviour (specified); D, Development; EG, egg maturation; F, Fecundity; L, Longevity; OF, offspring traits (specified); P, Predation rate; S, sex-ratio; r_m, Intrinsic rate of increase; * Significant effects; [a], avoidance without contact; [b], avoidance after contact. Table modified from Garcia (2011)

Species	Class of pesticide	Active ingredient	Active ingredient concentration	Exposure (individuals exposed)	Sublethal effect	IOBC classification	Reference
Chrysoperla externa	Pyrethroid Pyrethroid Pyrethroid/ Organophosphorus	Fenpropathrin Zeta-cypermethrin Deltamethrin/ Triazophos	$0.40\ g\ L^{-1}$ $0.05\ g\ L^{-1}$ $0.02/0.70\ g\ L^{-1}$	Spraying (pupae) Spraying (adults)	D, F, OF(viability)	Harmless (1) Harmless (1) Slightly harmful (2)	Andrea *et al.*, 2013
Chrysoperla externa	Pyrethroid	Betacyfluthrin	$0.013\ g\ L^{-1}$	Spraying (eggs)	S, D, F, OF (viability)	Harmless (1)	Silva *et al.*, 2012
Chrysoperla externa	Pyrethroid	Permethrin	$0.08\ g\ L^{-1}$	Contact with residues (third instar larvae)	B (Walking, repellence* and irritability*)	-	Cordeiro *et al.*, 2010
Chrysoperla carnea	Pyrethroid	Deltamethrin	$100\ mg\ L^{-1}$	Spraying (eggs) Spraying (pupae) Contact with residues (first instar larvae) Contact with residues (adult)	D, F D, F D, L, F, OF (viability) L, F, OF(viability)	Harmless (1)	Giolo *et al.*, 2009
Chrysoperla carnea	Chloronicotinyl	Imidacloprid	$12\ mg\ L^{-1}$	Contact with residues (first instar larvae)	D*, L, F, OF (viability)	-	Golmohammadi *et al.*, 2009
Chrysoperla carnea	Chloronicotinyl	Imidacloprid	$147\ \mu g\ ml^{-1}$	Contact with residues (first instar larvae)	D, L, F, OF (sex-ratio), r_m	Harmless (1)	Rezaei *et al.*, 2007
Chrysoperla carnea	Chloronicotinyl Pyrethroid	Imidacloprid Pyrethrin + PBO (piperonyl butoxide)	$75\ ml\ p.c\ hl^{-1}$ $200\ ml\ p.c\ hl^{-1}$	Spraying prey (second instar larvae)	D, F, OF (viability), P*	-	Huerta *et al.*, 2004
Chrysoperla externa	Pyrethroid	Fenpropathrin	$0.09\ g\ L^{-1}$	Spraying (eggs) Spraying (first, second and third instar larvae)	D D*, OF (viability)	Harmless (1)	Carvalho *et al.*, 2002

prior to the experiment.

Chemicals tested

The insecticides Imidacloprid (Confidor Classic [200 g a.i. L^{-1}], Bayer) and Deltamethrin (Decis [25 g a.i. L^{-1}], Bayer) were used in these essays. For each insecticide, fresh solutions diluted with distilled water were prepared at the doses recommended by the manufacturer for the control of aphids and/or whiteflies. Solutions with 100 mg a.i. L^{-1} and 12.5 mg a.i. L^{-1} respectively, corresponding to their respective label rate, were prepared in distilled water. The food source, *E. kuehniella* eggs (≤ 5 days) sterilized with UV radiation, was fixed to an egg card with distilled water and then treated by a spray of either pesticide solution or distilled water (control), using a Potter Tower at a 2 bar pressure with a volume of 6 ml. This resulted in a homogeneous spray coverage of 9.52 ± 2.17 L (mean ± S.D.) (Garcia *et al., 2006) of flui*d per cm^2, corresponding to a pulverisation pressure of 1000 L/ha. The egg card, containing ≈ 500 eggs, was then placed under a fume hood for at least 2 h or until dry, prior to be inserted in the vial containing the larvae.

Treatments

The eggs were presented to the predator on egg cards, with around ≈ 500 treated *E. kuehniella* eggs per card; one card was introduced within each vial containing a starved L_3 and left there for 24 h. For each treatment, three groups of 10 individuals randomly chosen from different cohorts were used, for a total of 30 replicates per treatment.

Statistical analysis

A One-way ANOVA (factor pesticide treatment with 3 levels: P1 for Imidacloprid; P2 for Deltamethrin; C for the Control), followed by Tukey HSD multiple comparison tests (α= 0.05), was performed on data (SPSS®, 2009), except for sex-ratio which was compared through a Chi-square test. Interpretation of data was based on Zar (1996).

RESULTS

The ANOVA revealed a significant difference for L3 weight gain between the treatments ($F_{2,57}$ =16.908, P = 0.000) (Table 2a); Deltamethrin led to a lower weight gain than control (P = 0.000) and Imidacloprid treatments (P = 0.006). The weight at the pupae stage was not significantly different between treatments ($F_{2,86}$ =3.070, P = 0.052) within the 5% *P error* but post-hoc comparisons revealed that it was lower for Deltamethrin treatment when compared to control (P = 0.045). Then later during the life cycle, the weight gain from pupae to adult was significantly lower for Imidacloprid (P = 0.045).

For the sex-ratio, which is presented as the proportion of females in the total population, no significant differences were found between Imidacloprid and control (Chi^2= 0.703, P = 0.402) while Deltamethrin resulted in a significantly lower amount of adult females than Imidacloprid (Chi^2= 9.174, P = 0.02) and control (Chi^2= 4.748, P = 0.029) treatments. Deltamethrin led to a sex-ratio of 82.1% of male individuals when adult stages were sexed.

DISCUSSION

Sublethal effects of pesticides can be investigated by measuring weight, larval developmental variation and morphology changes in adults (Desneux *et al.*, 2007). In these essays, significant differences were found in the weight of

TABLE 2. Developmental (**a**) and life history (**b**) parameters, of *Chrysoperla agilis* fed on *Ephestia kuehniella eggs treated with Imidacloprid (P1), Deltametrin (P2) and distilled water (Control) at 25 °C. For each treatment, an initial number of 30 eggs were used (3 replicates X 10 individuals). Total number of individuals (N) included in the analysis between parentheses.* **a.** Duration and weight of developmental stages expressed in terms of X ± SE, and results of the ANOVA conducted on the data. For each treatment, means within a row followed by different letters are significantly different. **b. Mortality and malformation rates and sex ratio. For each treatment, means within a row followed by different letters are significantly different (chi^2 test).**

a. Developmental parameters	**Treatment**			ANOVA parameters
	P1	P2	Control	
Third instar (L3) developmental time (days)	4.10±0.06 (N = 29)	4.10±0.06 (N = 30)	4.07±0.09 (N = 30)	$F_{2,86}$ = 2.725 P = 0.071
Larval developmental time (days)	9.10±0.06 (N = 29)	9.30±0.09 (N = 30)	9.07±0.09 (N = 30)	$F_{2,86}$ = 2.725 P = 0.071
L3 weight variation (mg)	4.17±0.46b (N = 19)	2.52±0.30a (N = 20)	5.43±0.31c (N = 21)	$F_{2,57}$ = 16.908 P = 0.000*
Pupae weight (mg)	8.88±0.34 (N = 29)	8.24±0.21a (N = 30)	9.18±0.27b (N = 30)	$F_{2,86}$ = 3.070 P = 0.052
Weight variation from pupae to adult (mg)	1.72±0.28a (N = 20)	2.02±0.21 (N = 17)	2.54±0.22b (N = 20)	$F_{2,54}$ = 3.059 P = 0.055
Initial adult weight (mg)	6.48±0.24 (N = 20)	6.21±0.22 (N = 17)	6.83±0.28 (N = 20)	$F_{2,54}$ = 1.454 P = 0.243
Final adult weight (mg)	11.82±1.23 (N = 9)	11.80±2.15 (N = 3)	10.76±1.67 (N = 8)	$F_{2,17}$ = 0.155 P = 0.857
Adult weight variation (mg)	5.22±1.11 (N = 9)	5.77±2.11 (N = 3)	4.97±1.48 (N = 6)	$F_{2,15}$ = 0.053 P = 0.948
b. Life history parameters				
Mortality (%)	31.0% (N = 20)	43.3% (N = 17)	33.3% (N = 20)	
Malformations (%)	5.0% (N = 20)	11.8% (N = 17)	20% (N = 20)	
Sex-ratio	0.58b (N = 26)	0.18a (N = 28)	0.48b (N = 25)	

pupae and adult stages. Weight is overall a fitness factor and for the majority of arthropod species, females weight more than males (Ghiselin, 1974). This common trend among insects is most frequently attributed to fecundity selection favouring large females that can lay larger or more eggs than small females (Honek, 1993); female size is a main constraint on insect potential fecundity (Honek, 1993). Our results indicate a shifting in terms of individuals' weights from third instar larvae to pupae stage, first being lower for Deltamethrin then later in the development for Imidacloprid. Although not significant, we can see a trend in the remaining parameters for the Deltamethrin treatment, with a longer L3 developmental time, lower pupae weight, lower initial adult weight.

At the adult stage, both pesticides end up not being significantly different from control, leading us to believe on some tendency to recover from the impact of the treatment. When we take into account the biased sex-ratio towards males of the group under Deltamethrin, this shifting is even more pronounced as fewer adult females were included in the trial and in general males weight less than females. This may indicate a recovery capacity in this group.

However, Deltamethrin treatment resulted in a high mortality rate among female individuals after the ingestion of sprayed prey at the larval stage. This indicates that the use of Deltamethrin on crops where *C. agilis* are used as biocontrol agents is not advised, as it would lead to a significant decrease in females' abundance. Cordeiro *et al.* (2010) also reported high short-term mortality of lacewing larvae from two different species when treated with another pyrethroid, Permethrin. This is not particularly surprising as pyrethroids are broad-spectrum compounds and generally assessed as harmful to natural enemies of agricultural systems (Croft, 1990).

A factor to be taken into account is insecticide repellence as in agricultural settings, for it may reduce the population of non-target species in the area by stimulating its dispersal away from it (Cordeiro *et al.*, 2010), and therefore minimizing insecticide exposure when compared to what is observed in laboratory conditions. In this regard, preference tests where the studied individuals could choose between treated and untreated prey, would offer more understanding.

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