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Adverse effects of soil applied insecticides on the predatory coccinellid *Hippodamia undecimnotata* (Coleoptera: Coccinellidae)

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ABSTRACT

We studied, under laboratory conditions, the possible effects of sublethal doses of two soil applied insecticides (carbofuran and imidacloprid) on development, survival and fecundity of the predator *Hippodamia undecimnotata*. For studies, predator fed upon *Aphis fabae* that was reared on *Vicia faba* plants treated with the systemic carbofuran and imidacloprid. Survival of immature stages in insecticides treatments (67.6% and 52.2%, for carbofuran and imidacloprid, respectively) was lower than control (77.4%). Both insecticides did not affect significantly total immature developmental time, while carbofuran caused a significant reduction of adult weight. Adult average longevity was significantly higher for the control than the insecticides treatments. Moreover, females oviposited fewer eggs in both insecticide treatments than the control (33% and 55% reduction in average fecundity for imidacloprid and carbofuran, respectively). Population increase parameters were also adversely affected by insecticides application. The importance of the adverse effect of sublethal doses of systemic insecticides on designing and management of insects' pests are discussed.

1. Introduction

Coccinellids are important predators of aphids, coccids and spider mites (Iperti, 1999). However, their conservation in agroecosystems is limited by the extensive use of insecticides. Predatory coccinellids are likely to be exposed to a great number of insecticides while foraging in the field. Coccinellid predators can be exposed to insecticides directly during pesticide applications or by contacting pesticide residues while foraging on sprayed surfaces. Moreover, predators may be affected indirectly by consuming prey exposed to insecticides.

The direct contact effects of insecticides on coccinellid predators have been studied extensively, as have a number of lethal (mortality) and sublethal (developmental time, fecundity, locomotory behavior) effects on these predators (Olszak, 1999; Vincent et al., 2000; Youn et al., 2003; Lucas et al., 2004; Galvan et al., 2005; Bozsik, 2006). Soil applied systemic insecticides are generally absorbed by plant tissue and are therefore assumed to have few negative effects on insect predators (Pflüger and Schuck, 1991; Mizell and Sconyers, 1992; Ishaaya and Horowitz, 1998). Nevertheless, it is possible for a predator to acquire some systemic insecticides via their prey, as insecticides pass to plant tissues and via the plant sap pass to the herbivores. This may have a vital adverse impact on the population dynamics of a predator. The adverse effects of systemic insecticides applied in soil have been studied mainly for heteropteran predators that supplement their diets with plant sap (Torres and Ruberson, 2004) and to some extent for other predators and parasitoids feeding on plant pollen and floral and extrafloral nectar (Smith and Krischik, 1999; Stapel et al., 2000). However, a possible adverse effect of systemic insecticides on predatory insects, within a tritrophic context, has not been studied so far.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a broad spectrum systemic insecticide and nematicide. It widely used as an aerial or soil treatment against soil-dwelling and foliar feeding pests such as rootworms, wireworms, weevils, beetles, flea beetles, leafhoppers and aphids of certain field and row crops, the most common including potatoes, corn, rice, sugarcane, soybeans and wheat (Trotter et al., 1991). Soil applied carbofuran is absorbed by roots and is transported via plant fluids to the aerial parts of the plants (Caro et al., 1976). Insects are affected by contacting or ingesting carbofuran that acts as a cholinesterase inhibitor.

Imidacloprid is a systemic chloronicotinyl insecticide (1-[(6-chloropyridin-3-yl) methyl]-*N*-nitro-4, 5-dihydroimidazol-2-amine) that enters pest through via ingestion or direct contact. It acts by disrupting nicotinic acetylcholine receptors in the insect nervous system (Mullins, 1993). It is worldwide used on ornamentals, field crops and vegetables with application to seeds, soil and foliage for controlling many pests including aphids, scales insects, white flies, some Coleoptera, some Lepidoptera and Diptera. After

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soil application, imidacloprid is absorbed by roots and transported via xylem to the aerial parts of the plant (Horowitz et al. 1998; Ishaaya and Horowitz, 1998; Buchholz and Nauen, 2002).

Hippodamia (*Semiadalia*) *undecimnotata* (Schneider) (Coleoptera: Coccinellidae) is a common European species (Iperti, 1999). Females prefer to lay eggs on low plants (0–50 cm) infested with aphids, with preferences to plants of the Leguminosae family (Iperti, 1999). In Greece, it can be found on maize, cotton, alfalfa, tobacco and non-cultivated plants (Kavallieratos et al., 2004; Katsoyannos et al., 2005).

The aim of the present study was to investigate the effects of sublethal doses of soil applied imidacloprid and carbofuran on development; survival and fecundity of *H. undecimnotata*; and to estimate population increase parameters of the predator in relation to insecticides application.

2. Materials and methods

2.1. Insects

Laboratory cultures of *H. undecimnotata* were established from individuals collected in the middle of August 2006 from an alfalfa field in the area of Arta (Epirus, south-western Greece). Ladybird beetles were reared on *Vicia faba* L. (Leguminosae) broad beans infested with *Aphis fabae* Scopoli (Hemiptera Aphididae) held in an environmentally controlled room at 24 ± 1 °C, 70 ± 5% RH, and a photoperiod of 16:8 (L:D) h. F_3 progeny were used for the experiments. Aphid colonies were established from individuals collected from a commercial bean culture and they were reared on *V. faba* plants.

2.2. Insecticides application

Five broad beans were sown in pots (13.5 cm upper diameter, 7 cm base diameter, 12.5 cm height and 1 l volume) filled with a mixture of turf (black and white peat substrate, Floradur B fine, Floragard) and perlite (Perloflor, Protectivo Ltd.) in a proportion of 6 to 1 by weight. When plants reached the four-leaf stage, a 100 ml aqueous solution of insecticides was added to each pot. A dose of 0.0206 mg active ingredient/pot for imidacloprid (Confidor 20SC, Bayer AG) and 0.0355 mg of active ingredient/pot for carbofuran (Carbodan 35.5SC, Makhteshim Chemical Works LTD) was used. Based on the label rate per ha the doses that were used were 14 times lower than recommended one. These doses were chosen based on preliminary tests so that they did not significantly affect the development and survival of *A. fabae*. Twenty apterous adult aphids were placed on each plant, using a fine camel brush immediately after insecticide application. Ten days later the tops of the plants with aphid colonies bearing a sufficient number of aphids (approximately 150 aphis of all developmental stages) were cut and used for the experiments. Plants were used between the 10–15th day of the insecticide application.

2.3. Bioassay

Transparent 280-ml plastic cages (13.5 cm upper diameter, 7 cm base diameter and 12.5 cm height) were used for the experiments. An opening was made in the plastic cup through which a plant stem was placed. Plant parts were secured in place by molding with white plasticine around their stem and filling the opening. The upper opening of the plastic caps was closed with organdy gauze to ensure good aeration and humidity equilibrium. Cups containing the plants were fitted into a same plastic cap containing a sponge impregnated with water in a way that would allow the cut part of the plant stem to come in contact with the sponge.

For larval and pupal development studies newly hatched larvae were placed individually on *V. faba* plants colonized with *A. fabae*. Larvae were checked daily and their development and survival were recorded. Larvae were provided fresh prey daily until pupation (about 150 aphids of all developmental stages). Adults were sexed upon emergence and were weighed within 22–26 h after emergence. Adults were sexed by examination of the IX and X abdominal segments and moreover each adult after their death were dissected to confirm their sex. Thirty one, thirty three and forty six larvae were examined for control, carbofuran and imidacloprid, respectively.

In order to study adult longevity and fecundity pairs of newly emerged adults held individually in plastic cages and provided with food as described above. Pairs were observed periodically and only those that have been mated were used. Until onset of oviposition, pairs of adults were kept together but once oviposition was initiated males were placed in separate cages to reduce egg cannibalism. Every 10 days males were placed for 6 h into the females's cages to encourage copulation. Cages and plants were checked daily and adult mortality and number of eggs laid were recorded until all the tested adults died. Ten pairs of each treatment (control, carbofuran and imidacloprid) were studied.

Eggs were collected daily and placed in Petri dishes where they were observed daily and the incubation time and the egg hatching were recorded. Ten replicates (60–100 eggs per replicate) were performed for each treatment.

In order to determine the sex ratio, 100 eggs of each treatment were collected on different oviposition days and were placed in plastic cages in groups of 10. Larvae were provided with excess food until pupation. After adult emergence their sex was determined.

2.4. Statistical analysis

The differences in larval and pupal survival among treatments were determined by χ^2 test (Sokal and Rohlf, 1995). The effect of treatments on duration of larvae and pupal stages, adult weight, longevity and fecundity were evaluated by one-way analysis of variance and significantly different means were separated by LSD test. All analyses were conducted using the statistical package SPSS 14.0 (SPSS Inc., Chicago, IL, 2004). The data on immature survival and development were combined with the adult longevity and fecundity results to estimate population increase parameters. Net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), intrinsic rate of birth (b), intrinsic rate of death (d), doubling time (DT) and mean generation time (T) were estimated. Population parameters were estimated following the methods described by Carey (1994).

3. Results

3.1. Immature developmental time and survival

Rearing of *H. undecimnotata* larvae on aphids that had developed on plants whose soil had been treated with carbofuran and imidacloprid, had slight impact on the predator' developmental times (Table 1). Application of imidacloprid increased developmental time of female L_1 instars in relation to control (2.2 and 3.3 days for control and imidacloprid, respectively). An increase in developmental time was observed for L_2 females larvae when fed on carbofuran-treated plants (1.4 and 2.1 days for control and carbofuran). Pupal developmental time ranged from 5.4 to 5.9 days and was significantly shorter for females reared on treated plants than for females reared on control plant. However, the insecticides application did not significantly affected the total

Table 1
Larval and pupal developmental time and adult weight for *H. undecimnotata* fed on *A. fabae* colonies reared either on imidacloprid- and carbofuran-treated *V. faba* plants or non-treated *V. faba* plants

Treatment	Mean developmental time (days ± SE)						Mean adult weight (mg ± SE)
	L1	L2	L3	L4	Pupa	Total	
Male							
Control	2.5 ± 0.2a*	1.9 ± 0.1a	2.4 ± 0.2ab	5.4 ± 0.2a	5.4 ± 0.2a	17.6 ± 0.2a	17.7 ± 0.4a
Carbofuran	2.5 ± 0.2a	2.1 ± 0.1a	2.8 ± 0.1a	5.1 ± 0.2a	5.6 ± 0.2a	18.2 ± 0.2a	15.2 ± 0.4b
Imidacloprid	2.9 ± 0.3a	2.0 ± 0.2a	2.3 ± 0.2b	4.6 ± 0.2b	5.4 ± 0.2a	17.2 ± 0.4a	17.1 ± 0.7a
F^a	0.907	0.339	3.643	4.841	0555	3.305	7.514
P	0.415	0.715	0.038	0.015	0.580	0.050	0.002
Female							
Control	2.2 ± 0.2b	1.4 ± 0.2b	2.9 ± 0.2a	4.5 ± 0.2a	5.9 ± 0.1a	16.9 ± 0.3a	22.4 ± 0.6a
Carbofuran	2.1 ± 0.1b	2.1 ± 0.1a	2.7 ± 0.1a	5.0 ± 0.1a	5.4 ± 0.2b	17.3 ± 0.3a	18.6 ± 0.5b
Imidacloprid	3.3 ± 0.3a	1.6 ± 0.2b	2.3 ± 0.1a	4.4 ± 0.2a	5.4 ± 0.1b	17.1 ± 0.3a	21.9 ± 0.4a
F^a	10.771	4.892	2.878	2.911	5.573	0.441	14.225
P	0.000	0.013	0.070	0.068	0.008	0.647	0.000

*Means in the same column followed by the same letter are not significantly different ($P < 0.05$; LSD test).
[a] All *df* for males 2,30 and all *df* for female 2,35.

immature developmental time (larvae and pupae) that was 17.6, 18.2 and 17.2 days for males and 16.9, 17.3 and 17.1 days for females reared on untreated, carbofuran- and imidacloprid-treated plant, respectively.

A decrease in adult weight was detected when larvae of the predaceous beetles fed on aphids reared on carbofuran-treated plants compared to those reared on control plants (Table 1). Average adult male weight was 17.7 mg for control and 15.2 mg for carbofuran and average female weight was 22.4 mg for control and 18.6 mg for carbofuran. Imidacloprid did not significantly affect adult weight relative to controls.

The percentage immature survival (larvae and pupae) was 77.4, 67.6 and 52.2% for beetles reared on control, carbofuran- and imidacloprid-treated plants, respectively (Table 2). Survival of the immature predator stages fed on aphids from plants systemically treated with imidacloprid was significantly lower than that of control, and this difference resulted primarily from the high mortality in the first instars.

3.2. Adult longevity and fecundity

The soil application of systemic carbofuran and imidacloprid negatively affected the adult lifespan, pre-oviposition period and fecundity (Table 3). For females, the average longevity was 122.9 days for control and 79.8, 98 days for carbofuran and imidacloprid treatments, respectively. Reduced average longevity on insecticide-intoxicated prey was observed for males as well (104.3, 63.6 and 72.2 days for control carbofuran and imidacloprid, respectively). Survival of the adults reared on untreated plant was very high (100%) through the first 80 days, whereas survival of the carbofuran and imidacloprid-treated adults was much lower (Fig. 1). Pre-oviposition period was significantly shorter for the control females (6.9 days) than for both insecticide treatments (>8.4 days). Insecticide-treated females laid significantly fewer eggs than control females. The lowest eggs production was observed for females fed on carbofuran-treated plants (490.3 eggs per female). The average fecundity of females fed on imidacloprid-treated plants was 725.3 eggs per female while the average fecundity for control females was approximately 1089 eggs per female. The maximum daily egg production for control and imidacloprid-treated females was approximately 28 eggs per female while for that reared on carbofuran-treated plant did not exceed the 18 eggs per female (Fig. 1). Egg hatchability was lower for the insecticides treatment than for the control but these differences were not significant. Incubation time ranged from 3.2 to 3.3 days and no significant differences were found. Insecticides did not affect sex ration and the ratio males vs females was approximately 0.82 for all treatments (data not show).

Table 2
Percent larval and pupal survival for *H. undecimnotata* fed on *A. fabae* colonies reared either on imidacloprid and carbofuran-treated *V. faba* plants or non-treated *V. faba* plants

Treatment	L1	L2	L3	L4	Pupa	Larva	Total
Control	93.5a*	86.2a	100a	100a	96.0a	80.6a	77.4a
Carbofuran	81.8a	96.3a	100a	100a	88.5a	76.5ab	67.6ab
Imidacloprid	63.0b	100a	89.6a	100a	92.3a	56.5b	52.2b

*Means in the same column followed by the same letter are not significantly different ($P < 0.05$; chi-squared test).

3.3. Population parameters

The value of net reproductive rate was 214 for population fed on aphids of untreated plants while the values of the same parameter were much lower for predators fed on aphids of treated plants (56 and 62 for carbofuran and imidacloprid, respectively) (Table 4). Intrinsic rate of increase for control population was approximately 0.08 and 0.069 and 0.067 for populations fed on carbofuran and imidacloprid-treated plants. Mean generation times were 67.4, 58.2 and 61.5 for control, carbofuran and imidacloprid respectively. Doubling time of the control beetles was 8.7 days and doubling times for beetles in the carbofuran and imidacloprid treatments were 10.0 and 10.3 days, respectively.

4. Discussion

Our data demonstrate clear adverse effects of soil applied insecticides on *H. undecimnotata* immature and adult performance. Predation on aphids fed on systemically treated plants, resulted in a wide range of effects on predator, including the simultaneous manifestation of multiple sublethal effects. In addition to high larvae mortality, the exposure to insecticides resulted in lighter adults that eventually laid a lower number of eggs and had shorter life span. However, the two insecticides affect the performance of the predator in different ways. Imidacloprid influenced larvae survival more than carbofuran while carbofuran influenced adult weight, life span and fecundity more than imidacloprid. The consequence of these effects was a substantial adverse impact on the population increase parameters of the predator. Although the significance of demographic analysis, is recognized as a tool for the better evaluation of insecticides toxicity against arthropods, few studies have been performed on these topics concerning the effects

Table 3
Longevity, fecundity and fertility of *H. undecimnotata* fed on *A. fabae* colonies reared either on imidacloprid- and carbofuran-treated *V. faba* plants or non-treated *V. faba* plants

Treatment	Longevity (days) (mean ± SE)		Pre-oviposition period (day ± SE)	Eggs per female (mean ± SE)	Eggs hatched (% ±S)	Ovipositing female% (T/O)
	Female	Male				
Control	122.9 ± 7.7a*	104.3 ± 4.5a	6.9 ± 0.3a	1089.1 ± 69.2a	70.2 ± 6.0a	100 (10/10)
Carbofuran	79.8 ± 12.3b	63.6 ± 9.6b	8.4 ± 0.2b	490.3 ± 122.6b	56.0 ± 5.5a	100 (10/10)
Imidacloprid	98.0 ± 4.4ab	72.2 ± 10.7b	9.8 ± 0.3c	725.3 ± 102.1b	58.2 ± 4.9a	90(10/9)
F	6.108	6.035	24.090	9.025	1.962	
df	2,27	2,27	2,26	2,27	2,26	
P	0.006	0.007	0.000	0.001	0.161	

*Means in the same column followed by the same letter are not significantly different ($P < 0.05$; LSD test).

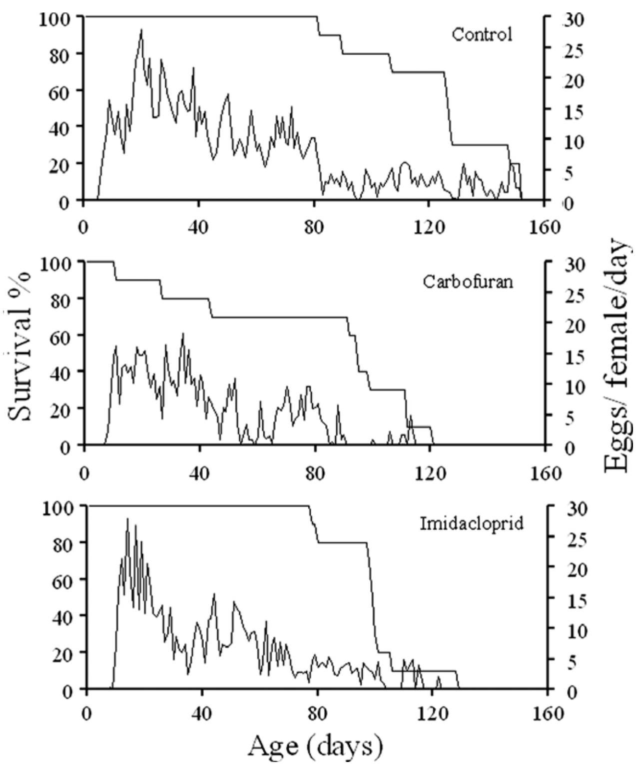

Fig. 1. Survival and age specific oviposition of females *H. undecimnotata* fed on *A. fabae* colonies reared either on imidacloprid- and carbofuran-treated *V. faba* plants or non-treated *V. faba* plants.

Table 4
Population parameters of *H. undecimnotata* fed on *A. fabae* colonies reared either on imidacloprid- and carbofuran-treated *V. faba* plants or non-treated *V. faba* plants

Parameter	Control	Carbofuran	Imidacloprid
Net reproductive rate (R_0)	214.08	56.49	61.99
Intrinsic rate of increase (r)	0.080	0.069	0.067
Finite rate of increase (λ)	1.083	1.072	1.069
Intrinsic rate of birth (b)	0.118	0.139	0.152
Intrinsic rate of death (d)	0.038	0.063	0.085
Doubling time (DT)	8.7	10.0	10.3
Mean generation time (T)	67.4	58.2	61.5

of insecticides in population dynamics of predatory insects (Stark and Banks, 2003). In the limited studies where effects of insecticides on population increase parameters of predatory insects is reported, (Rezaei et al., 2007) exposure of larvae of *Chrysoperla carnea* (Stephens) to imidacloprid residue had not a significant impact on life table parameters for the predator. Even though, the direct comparison of the result may not be feasible due to the differences in methodology and insect species, it indicates the different effects that insecticides may have under different ways of predatory insect exposure.

There is a growing literature demonstrating sublethal effects of imidacloprid or carbofuran on invertebrates (Kerns and Stewart, 2000; Widiarta et al., 2001; James, 2003; Decourtye et al. 2004; Capowiez et al., 2006), and there are cases where no adverse effects were observed with sublethal doses of imidacloprid (Mahdian et al., 2007). Adverse effects of systemic insecticides applied to soil and acquired by plants have been reported mainly for heteropteran predators that supplement their diets with plant sap (Torres and Ruberson, 2004). However, coccinellid predator *Coleomegilla maculate* (DeGeer), a facultative pollen feeder, when confined with inflorescences of plants in imidacloprid-treated soil had reduced mobility and survival and increased flip time (amount of time required for the beetle to roll onto their ventral surface after being rolled onto their backs) and pre-oviposition period (Smith and Krischik, 1999). In greenhouse experiments, survival of adult green lacewing, *C. carnea* was reduced after feeding on flowers from plants treated with a soil application of imidacloprid (Rongers et al., 2007). Stapel et al. (2000) found strong adverse effects on the survival and the foraging behavior of the parasitoid *Microplitis croceipes* Cresson when it was fed on extrafloral nectar of cotton plants treated with the systemic insecticides imidacloprid, acephate and aldicarb. The studies so far refer to toxicity effects of the insecticides to natural enemies by feeding on parts or secretions of soil-treated plants. In our case we demonstrated that both insecticides when applied to soil, even at doses that are not harmful to aphids, could have a clear negative impact on the population dynamics of their predator. Moreover, our data indicates that predator could reach soil applied insecticides through their prey. The similar result that soil applied insecticides can reach predators through their prey reported by James (2003) testing the toxicity of systemic imidacloprid against the predatory mite *Galendromus occidentalis* Nesbitt. However, in some field studies, soil application of imidacloprid and carbofuran had no adverse effects on predatory populations of some families including Coccinellidae (Asin and Pons, 1999; Albajes et al., 2003).

Our data showed that soil applied insecticides (imidacloprid and carbofuran) can affect negatively some life history parameters of predaceous coccinellid *H. undecimnotata*, after preying on insects that were reared on foliage of plants in treated soil. The results of our study showed that doses of insecticides that are sublethal to the aphid prey could adversely affect crucial parameters of predator's biology. The effects that soil applied systemic insecticides have to predator population dynamics may be of critical importance in the maintenance and reinforcement of integrated pest management programs or to the development of an effective biological control program against aphids. It should be noted here that the magnitude or the range of adverse pesticide effects on predatory insects may vary across plant type or insect taxa. When referring to non-target effects of insecticides it is rarely if ever mentioned the influence of sublethal doses to the population dynamics of the natural enemies. Our results show that this aspect could be of high importance and it should be included in risk analysis studies or in integrated pest management programs.

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