

EFFECTS OF SOME INSECTICIDES APPLIED IN SUBLETHAL CONCENTRATIONS ON THE SURVIVAL AND LONGEVITY OF *Aphidius ervi* (Haliday) (HYMENOPTERA: APHIDIIDAE) ADULTS

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ABSTRACT

The effects of four insecticides (dimethoate, pirimicarb, imidacloprid, and spinosad) applied in an ST4 Potter tower at sublethal concentrations (50% of those recommended commercially for aphid control) were studied in the laboratory on the aphidiid *Aphidius ervi* (Haliday) adults, an important parasitoid of the pea aphid *Acyrtosiphon pisum* (Harris). The most selective treatment on the hymenopteran was imidacloprid, followed in decreasing order by spinosad, pirimicarb, and finally dimethoate, which quickly eliminated the parasitoid and thus its capacity to produce progeny. Three toxicity groups were distinguished in the study. The least damaging treatment to adults of *A. ervi* was imidacloprid, followed by a group of medium toxicity made up of spinosad and pirimicarb, and lastly dimethoate, which was extremely toxic to *A. pisum*.

Key words: *Acyrtosiphon pisum*, *Aphidius ervi*, dimethoate, green pea aphid, imidacloprid, pirimicarb, spinosad.

INTRODUCTION

After years of trust in pesticides, natural enemies are recognized as essential pest control agents in the long run (Banken and Stark, 1998). These organisms are important in the dynamics of communities of arthropods. Natural control agents interact with their hosts in a cyclical homeostasis between their populations (Rodríguez, 1980). Parasitoids are different from predators in that the first spend most of their life inside their hosts, and thus are subjected to the same factors of mortality (Ives and Settle, 1996). Hymenopteran parasitoids are important in natural control of aphids, particularly pea and cereal aphids, and are used in biological control programs of them in diverse crops (Pungerl, 1984).

Although biological control is desirable, some pests having high reproductive rate and mobility as certain aphids, are very difficult to control only biologically, and require selective insecticides acting together with natural control (Stark and Rangus, 1994). Integrated pest management includes natural enemies, complemented with selective insecticides when necessary (Metcalf, 1982).

In Chile, the pea aphid *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae) is a primary pest on diverse legume crops (Prado, 1991), which is controlled often with systemic insecticides. The leaves of the host plants become twisted and deformed, flower shoots do not grow and buds become atrophied. Plants infested get yellowish, and sometimes die. Soft winters and moderate temperature favour heavy infestations. This aphid is also a vector of viral diseases, such as *Bean mosaic virus* (Artigas, 1994).

Some insecticides used against aphids are pirimicarb (carbamate: 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate), dimethoate, (organophosphate: *O,O*-dimethyl *S*-methylcarbamoylemethyl phosphorodithioate) and imidacloprid (neonicotinoid: (E)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine). Dimethoate is toxic to many insect pests and bees; pirimicarb poses a risk to these pollinators, and is a specific aphicide, not toxic to natural enemies of aphids. Imidacloprid is effective mainly against sap-sucking insects, and possesses low toxicity to natural enemies and a large residual period (Barberá, 1989). Spinosad, a mixture of spinosyns A and D, which confer it high toxicity against numerous insects, comes from fermentation of a soil bacterium, *Saccharopolyspora spinosa* Mertz & Yao. Differently to other physiologically comparable products, it acts onto a different site of neuromuscular action, which translates into a hyperexcitation which paralyzes the insect from neuronal fatigue (Thompson *et al.*, 1999).

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Although spinosad is not recommended as an aphicide (in Chile or elsewhere), it is used on crops against many pests. Many of these ecosystem harbour aphids, which justifies the study of its effects on both pests and their parasitoids. Today, more than studying the toxic effect of pesticides on pests, there is a trend to evaluate their innocuousness towards natural enemies (Banken and Stark, 1998).

Aphidius ervi (Haliday) (Hymenoptera: Aphidiidae) is a common parasitoid of *A. pisum* and other aphids (Cameron *et al.*, 1984; Prado, 1991; Bueno *et al.*, 1993; Sequeira and Mackauer, 1993; Christiansen-Weniger and Hardie, 1997). In Australia, *A. ervi* regulates density of *Acyrtosiphon* spp. on alfalfa (*Medicago sativa* L.) and other legume crops; parasitism increased from 1 through 90% the second year from its introduction (Milne and Bishop, 1987). In previous studies, Zuazúa *et al.* (2000) observed that the average of *A. ervi* adults treated with commercial dosages of the same insecticides included in this study did not vary significantly from that in the untreated control, and related the effect of sublethal concentrations on *A. pisum* with the preservation of *A. ervi* in the agroecosystem.

Although many insecticides exert good aphid control in the short run, the application of broad spectrum products has ended often with their natural enemies, and caused a surge of pests under that control (Bartlett, 1958; Kain *et al.*, 1976; Croft, 1989).

In general, adult hymenopteran parasitoids are at least as susceptible to contact with insecticides as their respective hosts. Thus, many adult parasitoids are destroyed during programs of control with non selective insecticides (Bartlett, 1958). Integrated pest management includes the action of natural enemies to reduce the density of the first to levels near the economic threshold, which allows relatively low population densities of pests, and with it, the continued action of their natural enemies. In the study of an insecticide, keeping in mind the preservation of these control agents, all sublethal effects have to be considered. Together with establishing the longevity of a parasitoid after application of an insecticide, it is necessary to evaluate how this will affect its progeny and descendants.

The objective of this research was to determine in the laboratory the possible sublethal effects of the insecticides dimethoate, pirimicarb, imidacloprid, and spinosad onto adults of *A. ervi*.

MATERIALS AND METHODS

This study was done in two stages, in the Laboratory of Toxicology, Facultad de Ciencias Pecuarias, Universidad de Chile, Santiago, Chile.

Rearing. The aphids and the parasitoid were collected from alfalfa in Santiago. Identification of both species was confirmed using keys (Stary, 1966; Mertins, 1985; Pike *et al.*, 1997). The parasitoid was verified also by comparison with specimens from colonies at the Laboratory of Chemical Ecology, College of Sciences, University of Chile, Santiago.

Colonies of *A. pisum* were established with neonate nymphs onto faba bean plants (*Vicia faba* L.) on 13 cm diameter pots containing plant litter, sand, and humus (1:1:1), covered with transparent PVC cylinders with the top covered with fine cloth, which were maintained at 20 °C. These cylinders were used both to rear the aphids and *A. pisum* on them, and allowed to obtain the parasitoid continuously starting with the specimens obtained on the alfalfa; the adult parasitoids were fed with 10% honey on cotton wicks (Bueno *et al.*, 1993). When the plants became deteriorate and the aphids tended to migrate, or their populations risked being maintained, new potted plants were added. Parasitism developed so continuously, allowing enough and safe material.

Application of insecticides. The concentrations applied corresponded to half the average of the range recommended commercially for each product (Table 1). Volumes applied onto standard glass Petri dishes were equivalent to 1000 L ha⁻¹.

Treatments. Treatments to adults of *A. ervi* were applied with a Potter tower, spraying 0.5 mL of each insecticide (all products less than 1 yr-old) diluted in water (at 12 psi) onto 9 cm diameter glass Petri dishes (63.2 cm²) at the concentrations indicated in Table 1. Once the treated surface dried, 23 parasitoids younger than 24 h of age were placed per dish, obtained from the rearing cylinders, and were maintained at room temperature during 1 h. To ensure no untreated areas in the dish, 0.5 mL of the corresponding preparation were sprayed on the internal

Table 1. Insecticides and concentrations applied onto adults of *Aphidius ervi* in Petri dishes.

Treatments	Concentrations applied in 1000 L water	
	Active ingredients	Commercial products
Dimethoate	15 000 mL	37 500 mL
Spinosad	3 000 mL	6 250 mL
Imidacloprid	4 375 mL	12 500 mL
Pirimicarb	4 375 g	8 800 g
Control	-	-

side of the Petri dish cover. The calculation of the amount of insecticide was done by surface.

A 5 x 2 cm ventilation hole (10 cm²) covered with fine cotton cloth was made on the Petri dish covers to avoid a lethal chamber effect. Dead specimens after 1 h exposure were counted and sexed by presence of the oviscapt on females. The survivors were counted, taken to clean glass vials with food (10% honey in water), and maintained at 20 °C; the survival in each vial was registered daily, retiring and sexing the dead individuals until the last microhymenopteran was dead. Control Petri dishes were applied just water. All surviving individuals were eliminated.

A completely random design was used with five treatments and four replicates. The results of survivorship (%) and longevity (d) of the wasp after 1 h exposure with the insecticides on treated Petri dishes were subjected to ANOVA analyses (Statgraphic 5.0 for MS-DOS), after normalization using the arcsin \sqrt{y} transformation when needed. Significant differences ($P \leq 0.05$) between treatments were separated by mean of Duncan (1955) multiple range tests

RESULTS AND DISCUSSION

In a previous experiment, topically applied (sprayed directly onto the insects) insecticide treatments on *A. pisum* at sublethal concentrations obtained the results of mortality presented in Table 2. In another previous test, the same insecticides and dosages were applied with a

hand sprayer onto faba bean plants. Once dried, leaflets treated were caged into ventilated Petri dishes together with 5 d-old *A. pisum* nymphs. Mortality results are presented in Table 2.

Sublethal dosages (50% of the average of the commercial range) had aphicide action on *A. pisum* (Table 2). The topical application of dimethoate, pirimicarb or imidacloprid caused 100% cumulative mortality at 48 h. The dry residues of spinosad caused only 57.5% mortality in that 48 h period, which reveals nonetheless its potential in integrated control of pests, as this lesser toxicity represents surviving hosts for *A. ervi* and avoids the disappearance of the parasitoid from an ecosystem exposed to more toxic insecticides.

Longevity of adults of *A. ervi*. In this study, the average life-span of the adult parasitoids in the untreated control was 9.64 ± 0.07 d (males: 9.95 ± 0.19 ; females: 8.55 ± 0.38), while in Ives and Settle (1996), they did not survive more than 5 d. This variation could be due to differences in feeding and handling of the parasitoids between both studies.

A proportion of 0.26 females per male was obtained when rearing the parasitoid, previously to the experiment, near to the 0.36 in Zuazúa *et al.* (2000), but different from the two observed by Sequeira and Mackauer (1993). Anyhow, to obtain an adequate number of females is fundamental to maintain the colony, thus pairing must be maximized, exposing the females to males during 2-4 d, to ensure mating and sufficient progeny (Christiansen-Weniger and Hardie, 1997).

Table 2. Effect of topical application and of dry residues of insecticides at sublethal concentrations (50% of commercial dosage) on mortality of *Acyrtosiphum pisum*.

Treatments	Concentrations ¹	Cumulative mortality (%) after application			
		1 h	24 h	48 h	72 h
Topical application onto aphids					
Dimethoate	37.50 mL	50.00b	100.00c	-	-
Spinosad	6.25 mL	52.17b	73.91b	95.65b	100.00
Imidacloprid	12.50 mL	75.00c	100.00c	-	-
Pirimicarb	8.80 g	72.28c	100.00c	-	-
Control	-	0.00a	0.00a	0.00a	-
Aphids confined onto dry residues on faba bean leaves					
Dimethoate	37.50 mL	-	100.00b	-	-
Spinosad	6.25 mL	-	33.75ab	57.50b	87.50b
Imidacloprid	12.50 mL	-	83.75c	100.00c	-
Pirimicarb	8.80 g	-	100.00c	-	-
Control	-	-	0.00a	0.00a	0.00a

¹In both studies, n = 23 individuals per treatment, with four replicates. Concentrations to apply of commercial products per 1000 L correspond to 50% the average of the range recommended commercially for each product, with an application volume of 1000 L ha⁻¹.

Means in a column with different letters are significantly different according to Duncan multiple range tests ($P \leq 0.05$).

Survival of adults of *A. ervi*. After 24 h exposure to the dry insecticide residues, insecticides caused the death of all parasitoids (1 h for dimethoate) (Table 3). In the study of Lankin *et al.* (1997), metamidophos (another phosphorous insecticide) affected also severely the survival of adults of *Diaeretiella rapae* (McIntosh) exposed to residues. In the studies of Roos and Melo (1976), dimethoate was very toxic to all stages of development of the coccinellids *Cycloneda sanguinea* (L.) and *Eriopis connexa* (Germar). These results agree with Bartlett (1963), who considered dimethoate as a medium to high toxicity product. Imidacloprid had an intermediate range, with an average survival similar to the control. Spinosad and pirimicarb were not different from the control.

Longevity of adults of *A. ervi* after 1 h exposure to contact with the insecticides. In general longevity, there were significant differences between treatments according to the toxicity of the insecticides evaluated (Table 3). Dimethoate was the most toxic insecticide, which at the end of the first day caused total mortality and did not allow to measure longevity of males or females. Next was pirimicarb followed by spinosad. Both of these treatments presented lesser toxicity than dimethoate, but at the same time greater ($P \leq 0.05$) than the control. Among the insecticides evaluated, imidacloprid caused the least effect on life-span of adults (males, females, and the average of both). The general means of longevity of males and females in the trials were not significantly different. Neither were differences when comparing the longevity of both sexes in all treatments through a t-Student test, Lankin *et al.* (1997) did not find also differences in susceptibility between adults of *D. rapae* of both sexes exposed to contact with fenvalerate and metamidophos. However, Sequeira and Mackauer (1993) observed lower mortality on males than females of *A. smithi* Shao-Rao on foliage treated with carbaryl; the active searching behaviour of females increased their exposure to the insecticide.

In this study there were differences between pirimicarb and imidacloprid, differing from the results of Zuazúa *et al.* (2003), who did not find differences between both compounds in a laboratory comparison which included also other insecticides (thiacloprid and azadirachtin). Differences in results may be due to different laboratory conditions.

Stark *et al.* (1995) found that diazinon (an organophosphate insecticide, comparable by its mode of action only to dimethoate) was more toxic in terms of survival of *A. ervi* adults than to its host *A. pisum*, while imidacloprid was more selective in favour of the parasitoid.

In the laboratory pirimicarb has shown a lesser toxicity than imidacloprid to adults of *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) (Cohen *et al.*, 1996). However, the high toxicity of imidacloprid observed in the field would be lower due to the possibility of applying it to the roots, thus reducing direct contact with the parasitoids.

Besides being unnecessary, wide action spectrum insecticides as dimethoate make collapse populations of beneficial insects; however, pirimicarb at $\frac{1}{4}$ the concentration recommended produces efficient control of aphids on cereals, and does not have considerable toxic effects on beneficial fauna (Cornale *et al.*, 1996).

In studies of toxicity of diverse insecticides, Roos and Melo (1976) observed a great selectivity of pirimicarb on diverse stages of development of two coccinellid beetles, while dimethoate was very toxic. Bartlett (1963) classified this insecticide as a medium to high toxicity product, and pirimicarb as a compound clearly selective to the same coccinellids. As generalist predators, coccinellids have a larger pool of detoxifying enzymes and cannot be compared with parasitoids, which are more specific and have a less diverse enzymatic pool. However, Summers *et al.* (1975) verified the innocuousness of pirimicarb to adults and larvae of coccinellids, *Chrysopa* sp. (Neuroptera), *Nabis* sp., *Orius* sp. (both Hemiptera), and also the aphidiid hymenopteran *A. smithi*.

Table 3. Adults of *Aphidius ervi* surviving after 24 h exposure to insecticides at sublethal concentrations onto a treated surface and longevity (\pm standard error) by sex and general average.

Treatments	Longevity (d) \pm standard error			
	Surviving adults	Males	Females	Average
Dimethoate	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
Spinosad	23.00 \pm 0.00c	6.89 \pm 0.15b	6.40 \pm 0.29c	6.78 \pm 0.05c
Imidacloprid	22.00 \pm 0.41b	8.70 \pm 0.30c	8.10 \pm 0.24d	8.47 \pm 0.22d
Pirimicarb	22.75 \pm 0.25c	6.64 \pm 0.19b	5.15 \pm 0.54b	6.31 \pm 0.16b
Control	23.00 \pm 0.00c	9.95 \pm 0.19d	8.55 \pm 0.38d	9.64 \pm 0.07e

Means in a column with different letters are significantly different according to Duncan multiple range tests ($P \leq 0.05$).

Based on laboratory studies, Patel *et al.* (1996) classified dimethoate as a highly toxic product (> 50% mortality) on *D. rapae* (McIntosh), an aphidiid parasitoid of the cabbage aphid *Brevicoryne brassicae* L.

In the laboratory, under a 16:8 h photoperiod, day and night temperatures of 20 and 10 °C, respectively, Ives and Settle (1996) found that the period between egg laying of *A. ervi* and the formation of the mummy lasts 8.1 ± 0.7 d. The parasitoid emerges in 6.1 ± 0.8 d, with a total period between egg laying and emergence of 14.7 ± 1 d. The adults are short lived and normally do not survive over 5 d.

When using insecticides, the relationship between the amount of toxicant and the unit of live weight is greater for smaller organisms, and often, the parasitoids are smaller than their hosts (Croft, 1989). Thus, many insecticides are more toxic to natural enemies than pests (Croft and Brown, 1975). Studies indicate that, besides their lethality, insecticides have sublethal effects on microhymenopterans (Liotta, 1978; Maniglia, 1978), and affect their longevity, fecundity, juvenile development, rate of parasitism, longevity, fertility, egg laying, reproduction, and locomotion, and also cause deformities (Theiling and Croft, 1987; Banken and Stark, 1998), and alter the searching capacity of hosts or refuges against predators (Hoy and Dahlsten, 1984).

The impact of the action of natural enemies on pests may increase with the use of selective insecticides more toxic to the aphids than to beneficial organisms (Syrett and Penman, 1980). The selectivity of a pesticide can be physiological, a product of differences in absorption, detoxification, and excretion (Stark *et al.*, 1995). There also exists an ecological selectivity, when the toxic elements do not reach the beneficial agent, but they do get to the pest in toxic concentrations (Roos and Melo, 1976). This laboratory study must be validated with a field one studying the interactions between *A. ervi* and *A. pisum* with the weather, foliage, selective forms of application, behaviours of search and migration, etc. Another research will have to adjust dosages of insecticides, to obtain an adequate relationship between effectiveness of treatment vs. parasitoid fecundity.

CONCLUSIONS

In conclusion, using sublethal dosages, three insecticide categories of toxicity in terms of reduction of longevity were distinguished toward *A. ervi* in this study. The most selective treatment was imidacloprid, followed by a group of medium toxicity formed by spinosad and pirimicarb, and last by dimethoate, which was extremely toxic to *A. pisum*.

RESUMEN

Efecto de algunos insecticidas aplicados en concentraciones subletales en la supervivencia y longevidad de adultos de *Aphidius ervi* (Haliday) (Hymenoptera: Aphidiidae). Se evaluaron en laboratorio los efectos de cuatro insecticidas (dimetoato, pirimicarb, imidacloprid, y spinosad) aplicados en torre Potter ST4 en concentraciones subletales (50% de las dosis comerciales recomendadas para el control de áfidos) sobre adultos del afídido *Aphidius ervi* Haliday, un importante parasitoide del pulgón de la arveja *Acyrtosiphon pisum* (Harris). El tratamiento más selectivo sobre el himenóptero fue imidacloprid, seguido en orden decreciente por spinosad, pirimicarb, y finalmente dimetoato, compuesto que eliminó rápidamente al parasitoide y su capacidad de producir descendencia. Se distinguieron tres grupos de toxicidad. El tratamiento menos dañino para los adultos de *A. ervi* fue imidacloprid; seguido por un grupo de selectividad media formado por spinosad y pirimicarb; y por último dimetoato, que fue extremadamente tóxico.

Palabras clave: *Acyrtosiphon pisum*, *Aphidius ervi*, dimetoato, imidacloprid, pirimicarb, pulgón verde de la arveja, spinosad.

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