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Effects of pirimicarb, buprofezin and pymetrozine on survival, development and reproduction of *Coccinella undecimpunctata* (Coleoptera: Coccinellidae)

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The effects of pirimicarb (a neurotoxin), buprofezin (an insect growth regulator) and pymetrozine (an antifeedant) on *Coccinella undecimpunctata* were assessed by studying the survival and development of all immature stages and the survival and reproductive performance of adults. Insecticides were sprayed at doses recommended by the manufacturers for the control of aphids and/or whiteflies. None of the three insecticides had a significant effect on the survival of *C. undecimpunctata* eggs. When sprayed on larvae, buprofezin significantly reduced survival to adulthood to $<33\%$, compared to $>45\%$ for the control and other insecticide treatments. Rates of adult survival, fecundity, and fertility, and the percentage of egg hatch, were not significantly different between control and insecticide treatments. Thus, larval stages were more susceptible to insecticides than were adults. In general, pirimicarb and pymetrozine had no adverse effects on immature or adult stages of *C. undecimpunctata*, and hence are suitable for IPM of sucking pests.

Keywords: *Coccinella undecimpunctata* L.; IPM; side-effects; buprofezin pirimicarb; pymetrozine

Introduction

Aphids and whiteflies are attacked by many species of predators that can act together to suppress or delay the outbreak of damaging populations (Sechser, Ayoub, and Monuir 2003). However, control of these pests, especially in greenhouses, usually depends on repeated use of insecticides. This can lead to problems of resistance (Mizell and Schiffhauer 1990), environmental contamination (Garrat and Kennedy 2006), and reduced populations of natural enemies resulting in pest resurgence or secondary pest outbreaks (Youn, Seo, Shin, Jang, and Yu 2003). It is necessary, therefore, to understand how these natural enemies as well as the pest are affected by insecticides (Tillman and Mulrooney 2000; Sechser et al. 2003; Youn et al. 2003; Liu and Stansly 2004; Desneux, Decourtye, and Delpuech 2007; Stark, Vargas, and Banks 2007b). A first approach is to determine the effects of the insecticides on the biological control agent in laboratory bioassays (Smith and Krischik 2000). Although the results may not reveal the effects under field conditions (Olszak 1999), laboratory-based direct spray techniques (i.e. acute toxicity tests) nevertheless

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can be used to simulate the maximum possible contact exposure of the insects to the chemical. If no harmful effects are observed, the insecticide can be considered compatible with the nontarget species (Schmuck, Storck-Weyhermuller, Ufer, and Waltersdorfer 1997; Smith and Krischik 2000; Tillman and Mulrooney 2000; James 2003).

Predacious lady beetles are one important group of aphid and/or whitefly predators (Hodek and Honek 1996; Dixon 2000). Coccinellid susceptibility to chemicals varies with the species and the type of insecticide (Olszak, Pawlik, and Zajac 1994; Candolfi et al. 1999), as well as with the nature of exposure (Banken and Stark 1998; Grafton-Cardwell and Gu 2003). Even when an insecticide does not kill the predator, it may have multiple sublethal effects, including shortened longevity (Liu and Stansly 2004), reduced fecundity and fertility (Banken and Stark 1998; Liu and Stansly 2004; Galvan, Koch, and Hutchison 2005), prolonged developmental rates (Galvan et al. 2005) or pre-oviposition periods (Liu and Stansly 2004), decreases in weight gain (Galvan et al. 2005), mutations in the offspring (Olszak et al. 1994; Banken and Stark 1997, 1998) and changes in behaviour (Wiles and Jepson 1994; Provost, Coderre, Lucas, and Bostanian 2003; Singh, Walters, Port, and Northing 2004; Stark, Banks, and Acheampong 2004). By ignoring sublethal effects, toxicological studies that evaluate only lethal outcome may underestimate the negative impacts of insecticides on natural enemy populations (Stark and Wennergren 1995; Stark and Banks 2003; Galvan et al. 2005; Stark, Sugayama, and Kovaleski 2007a; Desneux et al. 2007).

Although studies have been conducted to test for sublethal effects of different insecticides on coccinellids, few of these have explored how such effects vary among developmental stages (Banken and Stark 1997, 1998; Olszak, Pawlik, and Zajac 1994; Olszak 1999). Instead, most such studies are limited to only a single developmental stage (e.g. Mizzell and Schiffhauer 1990; Kalushkov 1999; Liu and Standsly 2004). Consequently, the negative impact of insecticides on populations may often be underestimated, because under field conditions individuals of varying developmental stages may be present and hence may differ in how they are affected by insecticide application (Stark and Wennergren 1995; Olszak 1999).

Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) is a euryphagous predator that feeds especially on aphids (Raimundo and Alves 1986; Hodek and Honek 1996) and can act as an effective biological control agent against these pests (e.g. El Hag 1992; Zaki, El-Shaarawy, and Farag 1999). Although *C. undecimpunctata* has a large geographic range (Frazer 1988), few studies have assessed the susceptibility of this species to insecticides (Salman and Abd-el-Raof 1979; Lowery and Isman 1995; Schroeder, Baird, Upritchard, and Simeonidis 1996; Omar, El-Kholy, and Tohamay 2002). To further the development of integrated pest management (IPM) against sucking pests, we evaluated the lethal and sublethal effects on *C. undecimpunctata* of three insecticides used for control of aphids and/or whiteflies on horticultural crops, such as cabbage (*Brassica oleracea*) and sweet potato (*Ipomoea batatas*). These three insecticides, pirimicarb (a neurotoxin), buprofezin (an insect growth regulator) and pymetrozine (an antifeedant), have not been tested previously for their effects on *C. undecimpunctata*. The insecticides were selected based on their current and potential use for the management of sucking pests (Jansen 2000; James 2002; Liu and Stansly 2004), and also because they

represent distinct chemical families. In addition, these pesticides are considered selective to some beneficial insects, such as Cybocephalidae, Coccinellidae, Syrphidae, Chrysopidae (Erkiliç and Uygun 1997; Jansen 2000; James 2002), and are recommended as suitable for IPM by the manufacturers. The effects of these three insecticides on *C. undecimpunctata* were evaluated in the laboratory by assessing the survival and development of all preimaginal stages and, the survival and reproductive performance of the adults.

Materials and methods

Insects

Adults of *C. undecimpunctata* were collected in Sta. Maria Island, Azores, Portugal, early in the summer. Lady beetles were reared in net cages ($50 \times 50 \times 50$ cm), at $25 \pm 1^{\circ}$C, $75 \pm 5\%$ RH and a photoperiod of 16L:8D, using fluorescent lamps (Sylvania Standard F36W/133). The lady beetles 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. Eggs and larvae produced by these adults were used for experiments.

Insecticide experiments

The lethal and sublethal effects of the following insecticides on all preimaginal stages and adults of *C. undecimpunctata* were evaluated under laboratory conditions: Pirimicarb (Pirimor G, WG [0.375 g(AI)/L], Syngenta), buprofezin (Applaud, WP [0.125 g(AI)/L], Syngenta) and pymetrozine (Plenum, WP [0.2 g(AI)/L], Syngenta). 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.

Prior to the insecticide treatments, preimaginal stages or adults of *C. undecimpunctata* were placed into plastic Petri dish bottoms (150×15 mm) and held for 30 min at 15°C to reduce the insects' mobility. Preimaginal stages were sprayed individually, while treatments with the adults were carried simultaneously on one male and one female (one couple). Individuals were sprayed with 6 mL of the aqueous suspension of the insecticide or distilled water (control), using a Potter's Tower apparatus (Burkard, Rickmansworth, UK) at 2 kPa. This resulted in an homogeneous spray coverage of 9.52 ± 2.17 L (mean $\pm$ S.D.) of fluid per cm^2, corresponding to a pulverisation pressure of 1000 L/ha.

All bioassays were performed at $25 \pm 1^{\circ}$C, $75 \pm 5\%$ RH and a photoperiod of 16L:8D using fluorescent lamps (Sylvania Standard F36W/133).

Bioassays of preimaginal stages

Eggs were sprayed within 12 h of being laid; first to fourth larval instar and pupae were treated 12–24 h after molting to the tested stage. After spraying, insects were transferred to separate untreated cylindrical plastic boxes (diameter: 5 cm; height: 3 cm) and fed *ad libitum* with a mixed diet of *A. fabae*, *M. persicae* and multiflower

bee pollen. Each experimental set was carried out to test the effects of pirimicarb, buprofezin, pymetrozine and distilled water (control) on the survival rate and development times of each preimaginal stage of *C. undecimpunctata.* To estimate these parameters, preimaginal stages were observed twice a day (at 09:00 and 17:00 h). Ecdysis and mortality were recorded for larvae and pupae until adulthood. Larvae were considered dead when they failed to move their legs when stimulated with a fine brush. In the case of eggs and pupae, 1 week was allowed to enable them either to hatch or to reach adulthood; Cabral, Soares, Moura, and Garcia (2006) determined previously that 1 week exceeds the time required by *C. undecimpunctata* to complete development in these preimaginal stages. For each insecticide or control treatment and each developmental stage, the procedure was repeated for a total of at least 20 individuals randomly chosen from four cohorts. Thus, sample sizes were as follows: eggs ($n = 20$), first instar larvae ($n = 29$–32), second instar larvae ($n = 20$–21), third instar larvae ($n = 23$) and fourth instar larvae ($n = 27$–28). Within each treatment and for statistical analyses, each individual was considered a replicate.

Bioassays of adults

At 12–24 h after emergence, new adults were sexed and paired prior to spray treatments. After spraying, each couple was placed inside an untreated cylindrical plastic box (diameter: 5 cm; height: 3 cm) and fed *ad libitum* with a mixed diet of *A. fabae*, *M. persicae* and pollen. Adult lady beetles were observed twice a day to estimate pre-oviposition period, and male and female survival rates. Survival rates were measured as the percentage of males or females with longevity equal to or exceeding previously determined mean longevity for each sex: at 25°C, *C. undecimpunctata* adult females have a mean longevity of 30 days, while males live 44 days (Cabral et al. 2006). Fecundity (number of laid eggs), fertility (number of eggs with embryos) and percentage of egg hatch were also determined during the first 15 days after sexual maturation of the adults. The procedure was repeated for a total of 20 couples per insecticide or control treatments, randomly chosen from four cohorts. Within each treatment and for statistical analyses, each couple was considered a replicate.

Statistical analysis

Data were first described as regular means and standard errors. Analyses of variance (ANOVA) were conducted on all data, except for survival rates. Where statistical differences existed between treatment sets ($P < 0.05$), Fisher's Least Significant Difference (LSD) tests were used to separate the differing means (Zar 1996). Survival rates were analysed using Multiple Comparison test for Proportions, where significant results are represented by giving a $q_{0.05,\infty,4}$ value > 2.35 (Zar 1996). To reduce heterogeneity in variances, percentages of egg hatch were transformed by $\text{arcsine}\sqrt{(x)}$, and development time, pre-oviposition time, fecundity and fertility by $\sqrt{(x+0.5)}$ (Zar 1996). All analyses were performed using SPSS 12.0 Windows (SPSS, Chicago, IL, 2004).

Results

Bioassays of preimaginal stages

None of the three insecticides had a significant effect on the survival of *C. undecimpunctata* when sprayed on eggs ($\chi^2_{obs} = 7.67$, df = 3 & 80, $P = 0.053$). However, survival of first and second instars was reduced when eggs were treated with buprofezin (Figure 1A). When insecticides were sprayed on larval stages, buprofezin significantly reduced survival to adulthood compared to the other treatments: survival rates for the first, second, third and fourth instar were, respectively, 33% ($\chi^2_{obs} = 4.32$, df = 3 & 121, $P = 0.23$), 24% ($\chi^2_{obs} = 21.48$, df = 3 & 82, $P < 0.001$), 24% ($\chi^2_{obs} = 27.41$, df = 3 & 84, $P < 0.001$) and 33% ($\chi^2_{obs} = 52.33$, df = 3 & 109, $P < 0.001$) (Figure 1B–E). Buprofezin induces death associated with or following moulting to the next stage; thus negative effects were recorded in the preimaginal stage that followed the stage treated with insecticide (Figure 1). When buprofezin was sprayed on the fourth instar (Figure 1E), all individuals moulted to

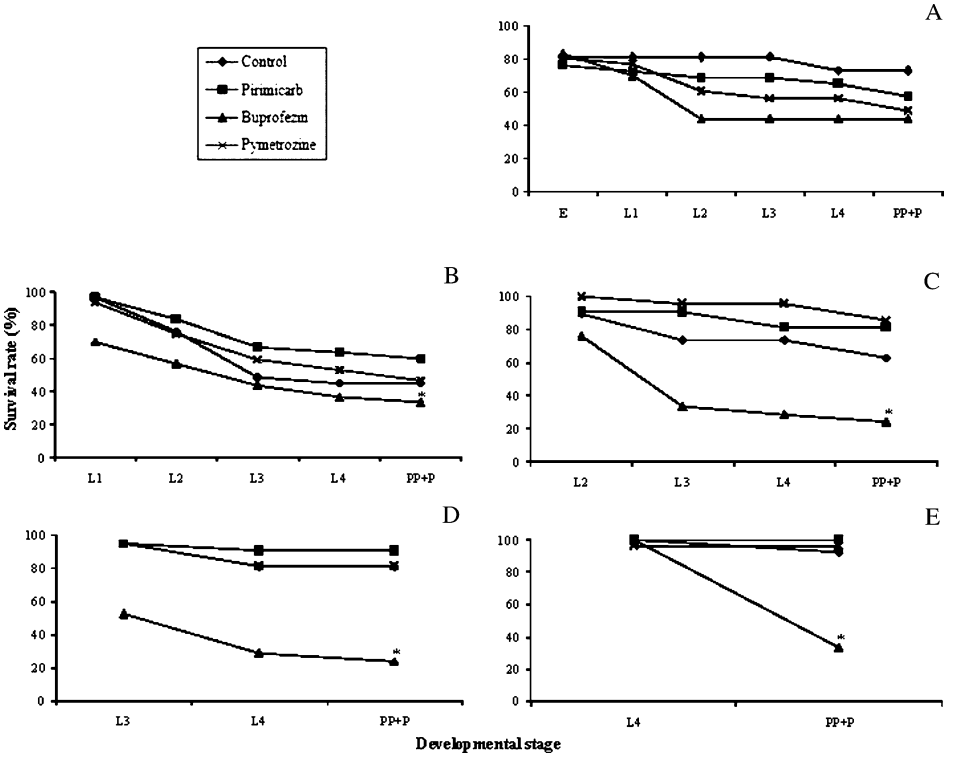

Figure 1. Cumulative survival rates of each preimaginal stage to adulthood of *Coccinella undecimpunctata*, when individuals were treated with pirimicarb, buprofezin, pymetrozine or distilled water (control group) at the following stages: A, eggs; B, first larval stage; C, second larval stage; D, third larval stage; E, fourth larval stage. Legend: W-egg; L_1, L_2, L_3 and L_4 – first, second, third and fourth larval stage, respectively; PP+P, prepupae + pupae. Within each graph, survival rates marked with an asterisk (*) are significantly different at $P < 0.05$ (Multiple Comparison for Proportions).

pupae; however, most of them failed to emerge as adults, or emerged as adults with teratogenic abnormalities (i.e. abnormal abdomen and deformed elytrae).

Survival of treated pupae was reduced by insecticide application (control = 80%; primicarb = 48%; buprofezin = 52% and pymetrozine = 54%), but not significantly ($\chi^2_{obs} = 6.558$, df = 3 & 101, $P = 0.09$).

Larvae took significantly longer to develop when eggs were sprayed with buprofezin ($F_{(3,75)} = 11.69$; $P < 0.001$), and when first instars were treated with pymetrozine ($F_{(3,105)} = 3.59$; $P = 0.016$) (Figure 2).

Bioassays of adults

Pymetrozine was the only insecticide that significantly increased the pre-oviposition period of *C. undecimpunctata* (Table 1). Fecundity, fertility and percentage of egg hatch were not significantly different between insecticide treatments and control. Although lower than the control, male and female survival rates were not significantly affected by the insecticide treatments (Table 1).

Discussion

As IPM has gained acceptance as a preferred approach of pest control, many efforts have been initiated to protect natural enemies by choosing selective insecticides (Croft 1990). Knowledge of the lethal and sublethal effects of insecticides on beneficial organisms is required for a complete analysis of their impact (Olszak 1999; Desneux et al. 2007).

Although considered selective to some beneficial insects (Erkiliç and Ungun 1997; Jansen 2000; James 2002; IOBC 2005) and recommended for IPM by the manufacturers, the insecticides tested in this study nevertheless had some adverse

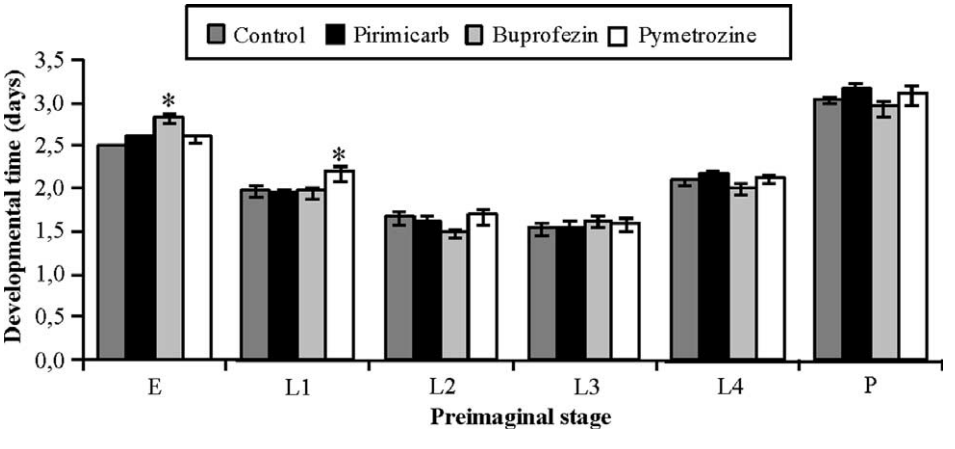

Figure 2. Developmental time (days ± SE) of different preimaginal stages of *Coccinella undecimpunctata,* when sprayed with pirimicarb, buprofezin, pymetrozine or distilled water (control group). Legend: E-egg; L_1, L_2, L_3 and L_4 – first, second, third and fourth larval stage, respectively; P, Pupae. Within a developmental stage, means in each column with an asterisk (*) are significantly different at $P < 0.05$ (LSD tests).

Table 1. Pre-oviposition period (days ± SE), fecundity (number of laid eggs ± SE), fertility (number of eggs with embryos ± SE), percentage of egg hatching (percentage of hatched eggs ± SE) and male and female survival rates (percentage of individuals that reached each sex mean longevity) of *Coccinella undecimpunctata* when adults were treated with pirimicarb, buprofezin, pymetrozine or distilled water (control group).

	Treatment				Statistical analyses
Biological parameters	Control	Pirimicarb	Buprofezin	Pymetrozine	ANOVA
Pre-oviposition period	3.47 ± 0.17 a	3.59 ± 0.17 a	3.41 ± 0.15 a	4.19 ± 0.29 b	$F_{(3,70)} = 2.96$; $P = 0.037$
Fecundity	440.6 ± 61.53	413 ± 44.67	350.16 ± 45.94	417.76 ± 33.04	$F_{(3,70)} = 0.88$; $P = 0.457$
Fertility	383.25 ± 40.75	369.94 ± 53.89	340.79 ± 45.00	365.47 ± 34.77	$F_{(3,70)} = 0.20$; $P = 0.895$
Percentage of egg hatching	76.40 ± 0.22	83.33 ± 0.12	80.05 ± 0.13	80.06 ± 0.13	$F_{(3,70)} = 0.74$; $P = 0.533$
Survival rate					*Multiple Comparison for Proportions*
Males	78.57	72.73	62.50	57.14	$\chi^2_{obs} = 3.434$, df = 3 & 85; $P = 0.329$
Females	84.21	50.00	60.87	60.00	$\chi^2_{obs} = 5.389$, df = 3 & 84; $P = 0.145$

*Means in each row followed by a different letter are significantly different at $P < 0.05$ (LSD tests).

effects on *C. undecimpunctata*. Such effects varied with the mode of action of each insecticide and the developmental stage of the coccinellid. Pirimicarb and pymetrozine did not affect the survival of *C. undecimpunctata* preimaginal stages. On the other hand, buprofezin significantly decreased the survival of all preimaginal stages, except when sprayed on eggs. Similar results were observed by several authors when studying the larval survivorship of other coccinellids (Mendel, Blumberg, and Ishaaya 1994; Olszak et al. 1994; Grafton-Cardwell and Gu 2003; James 2004; Liu and Stansly 2004). The fourth instar was the least susceptible to buprofezin, as larvae pupated without any apparent difficulty; however, many individuals were not able to cast off the pupal skin or if so, the adults had some teratogenic abnormalities (abnormal abdomen and deformed elytrae). Other studies report similar teratogenic effects when testing buprofezin on *Chilocorus nigritus* (Fabricius) (Magagula and Samways 2000), azadiractin on *Coccinella septempunctata* (L.) (Banken and Stark 1997, 1998) and distinct IGRs on *Adalia bipunctata* (L.) and *C. septempunctata* (Olszak et al. 1994) (Coleoptera: Coccinellidae).

Buprofezin did not reduce egg hatch but affected the survivorship of hatching neonates (first and second instars). This result is consistent with results of James (2004) and Biddinger and Hull (1995) when testing buprofezin and tebufenozide (a chitin synthesis inhibitor) on *Stethorus punctum picipes* (LeConte) (Coleoptera: Coccinellidae).

According to Liu and Stansly (2004), chitin inhibitors can cause sterilisation of coccinelid adults; however, no significant effects of buprofezin on adult survival or progeny production were observed for *C. undecimpunctata*. Our results are similar to those of others who have studied the effects of buprofezin in other coccinellids (Magagula and Samways 2000; Grafton-Cardwell and Gu 2003).

The results of this study support the hypothesis of Youn et al. (2003) from their testing results for several (non-IGR) insecticides (e.g. acetamiprid, etofenprox), that preimaginal stages of coccinellids are more susceptible than adults. Olszak et al. (1994) also note such differential effect in their tests of IGRs (not including the one tested here) on *A. bipunctata* and *C. septempunctacta*, as expected due to the mode of action of IGRs.

Pirimicarb, a selective systemic insecticide with contact, stomach and respiratory action, has been found to be useful in the implementation of IPM against aphids (Syngenta 2004). Previous studies had shown that this insecticide is not toxic to several natural enemies (Sterk et al. 1999), including several coccinellid species (Dimetry and Marei 1992; Jansen 2000; Kennedy et al. 2001; James 2003). Consistent with these findings, our results showed that pirimicarb was unharmful to *C. undecimpuncata* preimaginal stages and adults.

Pymetrozine has a very selective mode of action, interfering with the nervous regulation of feeding behaviour of sucking pests such as aphids, whiteflies and planthoppers (Harrewijn and Kayser 1997; Sechser et al. 2003). Thus, negative effects on the parameters of *C. undecimpunctata* studied here were not expected. In fact, our results confirmed the selectivity of this insecticide. A single exception is the half-day increase of the pre-oviposition period of sprayed adults; however, this result should not be considered biologically relevant since this species has a long oviposition period (e.g. 25 days at 25°C when reared with *M. persicae*) (Cabral et al. 2006). Nevertheless, the increase in the pre-oviposition period could be explained by a possible reduction in adult food uptake, as a consequence of

insecticidal activity, resulting in a decrease of nutrients available for egg maturation. Such a hypothesis was also proposed by Galvan et al. (2005), after observing an increase in development time of early instars of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) when this species was sprayed with spinosad and indoxicarb.

In general, the positive features of pirimicarb and pymetrozine make these chemicals suitable tools for conserving valuable natural enemies, and efforts should be made to incorporate such selective insecticides in IPM programmes against aphids and whiteflies, particularly in horticultural crops (e.g. cabbage, sweet potato). Because these pests can occur together in these crops, the selectivity of such insecticides would allow the control of aphids by *C. undecimpunctata*. On the other hand, buprofezin decreased significantly the survival rates of all preimaginal stages (except for eggs) of *C. undecimpunctata*, although its adverse effects are still less harmful than those of many other groups of non-selective insecticides (e.g. Erkiliç and Uygun 1997; Lo 2004); Therefore, to test further the possibility of using buprofezin safely in IPM, additional field experiments are required to evaluate the effects of this IGR when applications are timed to coincide with *C. undecimpunctata* adult and egg stages, thereby avoiding the susceptible larval stages.

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