

## EFFECTS OF THE INSECT GROWTH REGULATOR FENOXYCARB ON IMMATURE *CHRYSOPERLA RUFILABRIS* (NEUROPTERA: CHRYSOPIDAE)

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### ABSTRACT

Fenoxycarb (Comply®), a juvenile hormone analog, was tested in the laboratory at three concentrations (0.1, 1.0 and 10.0 mg [AI]/l) for toxicity to eggs, three larval instars and pupae of *Chrysoperla rufilabris* (Burmeister). Significant effects of fenoxycarb on all immature stages of *C. rufilabris* were found and the degree of effects depends on the stages treated and the concentrations used. Fenoxycarb showed significant ovicidal effect on *C. rufilabris* eggs, with 66.7, 76.6 and 86.7% survival rates at 0.1, 1.0 and 10.0 mg (AI)/l, respectively. Lethal effects on larvae varied greatly with high survival rates when the larvae were treated in the first and second instars (76.7-86.7% and 90.0-93.3%, respectively), and low survival rates (6.7-16.7%) when third instars were treated. Mortality at the pupal stage ranged from 90.0 to 93.3%. Fenoxycarb significantly delayed the developmental times from the stage treated to adult emergence for all immatures of *C. rufilabris* that successfully developed to adults by 3.2-4.6, 2.3-3.0, 2.1-2.8, and 4.6-7.5 days when egg, first, second and third instars were treated, respectively, compared with water treated control. When treated as pupae, fenoxycarb had no significant effects on pupal development. Among the three larval stages, the third instar is the most susceptible and vulnerable stage. The compatibility of fenoxycarb in integrated pest management programs is discussed.

Key Words: Lacewing, predators, aphids, whiteflies, insect growth regulators, juvenile hormone

### RESUMEN

El fenoxycarb (Comply®), una hormona juvenil análoga fue probada en el laboratorio en tres concentraciones (0.1, 1.0 y 10.0 mg [AI]/l) para determinar la toxicidad a huevos, tres instares de larvas y pupas de *Chrysoperla rufilabris* (Burmeister). Se encontraron efectos significativos del fenoxycarb en todas las etapas inmaduras de *C. rufilabris* y el grado de los efectos depende de las etapas tratadas y las concentraciones usadas. Fenoxycarb mostró un efecto ovicida significativo sobre los huevos de *C. rufilabris*, con tasas de supervivencia de 66.7, 76.6 y 86.7% a las concentraciones 0.1, 1.0 y 10.0 mg [AI]/l, respectivamente. Los efectos letales sobre las larvas variaron mucho con tasas altas de supervivencia cuando las larvas fueron tratadas en el primer y segundo instar (76.7-86.7% y 90.0-93.3%, respectivamente), y tasas bajas de supervivencia (7.6%-16.7%) cuando el tercer instar fue tratado. La mortalidad en la etapa pupal varió entre 90.0 a 93.3%. El fenoxycarb retardó significativamente el periodo de desarrollo de la etapa tratada para la emergencia de los adultos en todos los inmaduros de *C. rufilabris* que se desarrollaron bien hasta adultos por 3.2-4.6, 2.3-3.0, 2.1-2.8 y 4.6-7.5 días cuando respectivamente fueron tratados huevo, primer, segundo y tercer instares, en comparación con los del grupo control que fue tratado con agua. Fenoxycarb no tuvo efectos significativos en el desarrollo pupal cuando las pupas fueron tratadas. Entre las tres etapas larvales, el tercer instar es la etapa más susceptible y vulnerable. Se discute la compatibilidad de fenoxycarb en los programas de manejo integrado de plagas.

Fenoxycarb, a non neurotoxic carbamate, exhibits juvenile hormone analog (JHA) activities on many insects despite being structurally dissimilar to insect juvenile hormone (JH) (Dorn et al. 1981; Grenier & Grenier 1993). It has shown JHA activities against insects in several orders including Lepidoptera, Coleoptera, Homoptera, Dictyoptera, Diptera, and Orthoptera (Masner et al. 1980; Grenier & Grenier 1993), but also exhibits some non JHA-specific effects on many insects (Retnakaran et al. 1985).

Integrated pest management (IPM) requires the use of selective pesticides that preserve the natural enemies of the pests. Therefore, knowl-

edge about the effects of pesticides on beneficials is indispensable. However, few studies have been conducted to determine the effects of fenoxycarb on nontarget beneficials. In a laboratory test, Bigler & Waldburger (1994) found that fenoxycarb at 150 mg [AI]/l was extremely toxic to the larvae of the common green lacewing *Chrysoperla carnea* (Stephens). Celli et al. (1997) investigated the activity of fenoxycarb on immatures of *C. carnea* and observed extremely high embryonic and larval mortality and numerous, often lethal, effects on larval development. Fenoxycarb was also found to be harmful (>50% mortality) to brown lacewing, *Micromus tasmaniae* (Walker), in the laboratory,

and slightly harmful (25-50% mortality) in the field (Rumpf & Penman, 1993; Rumpf et al. 1997, 1998). Hassan et al. (1991) reported that fenoxycarb (150 mg [AI]/l) was harmless or slightly harmful to 19 species of beneficial organisms, except for *C. carnea* and *Anthocoris nemoralis* (F.), and while it was harmful to these two predators in the laboratory, it was only slightly harmful to *C. carnea* in a semi-field test. Vogt (1994) found that fenoxycarb (100 mg [AI]/l) was moderately harmful to *C. carnea* larvae in the field.

The green lacewing, *C. rufilabris* (Burmeister), is a polyphagous predator in North America. *C. rufilabris* has potential as a biological control agent against several species of major insect pests, including silverleaf whitefly, *Bemisia argentifolii* Bellow & Perring (formerly known as *B. tabaci* [Gennadius]) (Breene et al. 1992; Legaspi et al. 1994; Nordlund & Morrison 1990); cotton aphid, *Aphis gossypii* Glover (Nordlund & Morrison 1990); Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Nordlund et al. 1991); tobacco budworm, *Heliothis virescens* (F.) (Nordlund & Morrison 1990); and corn earworm, *Helicoverpa zea* (Boddie) (Lingren et al. 1968). However, few studies on fenoxycarb have dealt with beneficial insects (Grenier & Grenier 1993), and no information is available in the literature on the effects on the green lacewing *C. rufilabris*.

The objective of this study was to determine the effects of fenoxycarb on all immature stages of *C. rufilabris*, the most common lacewing species preying on aphids, whiteflies and many other insects in south Texas and other southern states.

## MATERIALS AND METHODS

### *Chrysoperla rufilabris*

*Chrysoperla rufilabris* were obtained from a commercial supplier (Biofac Crop Care, Inc., Mathis, TX). Eggs (24-h old) were maintained in a growth chamber at  $20 \pm 2^\circ\text{C}$ ,  $50 \pm 5\%$  relative humidity and a photoperiod of 16:8 (L:D) h. For egg bioassays, the eggs were used as soon as they were obtained. To obtain desired stages of larvae and pupae, the eggs were allowed to hatch in the growth chamber. After the eggs hatched, the first instars were reared individually in clear plastic petri dishes (5.5 cm diam.  $\times$  1.0 cm deep) and were fed with *A. gossypii* feeding on cotton leaves. The larvae were reared until they developed to the desired instars. They were used  $\approx 24$  h after the previous molting. To obtain pupae, 2-day old cocoons (pupae) were used because it took about 1 to 2 days to further develop from prepupa to pupa (Legaspi et al. 1994).

### Treatments

Fenoxycarb (Comply® 40WP; Novartis, Greensboro, NC) was used at three concentrations: 0.1, 1

and 10 mg (AI)/l, and purified water (reverse osmosis, 7 ppm solids) was used as control. *Chrysoperla rufilabris* eggs, larvae or pupae were dipped in the dilutions or water for 3 s. The treated eggs, larvae or pupae were placed on paper tissues for  $\approx 2$  h to absorb extra dilution and air-dry. The insects were then individually placed in petri dishes. Each treatment had 10 replications, and each replication had 10-20 individuals. The larvae treated directly or hatched from treated eggs were fed with *A. gossypii* ad lib. Survival and development were recorded daily. We considered larvae dead if they no longer moved or twitched when being touched 2-3 times with a brush. Pupae were regarded as dead if they turned black, or showed signs of desiccation.

### Data Analysis

Percentage survival rates and developmental times (days) for all stages were analyzed using the general linear model (PROC GLM). Means were distinguished using the least significant difference test (LSD) after a significant *F*-test at  $P = 0.05$  (SAS Institute 1996).

## RESULTS

### Effects of Fenoxycarb on Survival of *C. rufilabris*

The survivorship of the different developmental stages of *C. rufilabris* treated at different stages is shown in Fig. 1. When eggs were treated, survival rates varied among the three fenoxycarb concentrations ( $F = 9.33-34.67$ ;  $df = 3, 8$ ;  $P = 0.0008-0.0054$ ) (Fig. 1A). The egg hatching rate was 66.7% at the highest concentration (10 mg [AI]/l), and 86.7% at the lowest rate (0.1 mg [AI]/l), compared with 100% in the water control. When first instars were treated, the survival rate decreased significantly at the two higher concentrations with 86.7% at 10 mg [AI]/l and 90.0% at 1 mg [AI]/l compared with 100% in the water control ( $F = 6.67$ ;  $df = 3, 8$ ;  $P = 0.0144$ ) (Fig. 1B). However, the survival rates were not significantly decreased in subsequent developmental stages. When second instars were treated, 93.3-96.7% developed to adults, with no significant difference among the 4 treatments ( $F = 0.44-0.67$ ;  $df = 3, 8$ ;  $P = 0.5957-0.7278$ ) (Fig. 1C). The most significant effects were found when third instars were treated (Fig. 1D). The survival rate of third instars (cocooning or pupation rate) was reduced significantly, and only 40.0-53.3% pupated compared with 100% in the water control ( $F = 5.67$ ;  $df = 3, 8$ ;  $P = 0.0222$ ). Subsequently, only 6.7-16.67% successfully developed to adults compared with 100% in the water control ( $F = 76.0$ ;  $df = 3, 8$ ;  $P = 0.0001$ ). Fenoxycarb had no significant lethal effects on pupae when they were treated at pupal stage ( $F = 0.89$ ;  $df = 3, 8$ ;  $P = 0.4872$ ) (Fig. 1E).

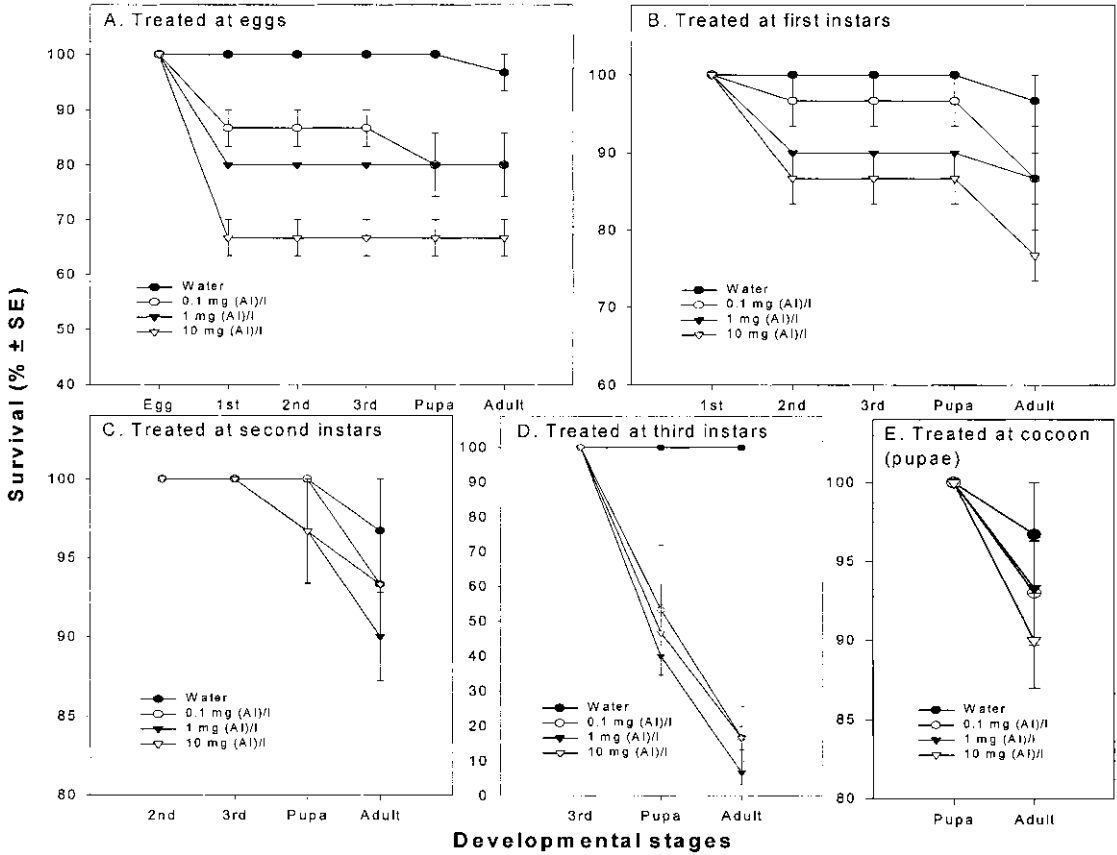


Fig. 1. Percentage of survival of eggs, larvae, and pupae of *Chrysoperla rufilabris* after treatment with three concentrations of fenoxycarb at different developmental stages.

Effects of Fenoxycarb on Development of Immature *C. rufilabris*

Developmental times of all stages of *C. rufilabris* after being treated with fenoxycarb are shown in Fig. 2 and Table 1. When eggs were treated, there were no significant effects on the developmental times of eggs at the two lower concentrations, but the developmental time of eggs was delayed 0.5 days at the highest concentration compared with that in the water control ( $F = 71.28$ ;  $df = 3, 88$ ;  $P = 0.0001$ ) (Fig. 2A). Significant development delays were also found in the third instar and pupal stage at all three of the concentrations. The developmental time of third instars was 2.3-2.8 days longer than that in the water control ( $F = 43.80$ ;  $df = 3, 86$ ;  $P = 0.0001$ ), and that of the pupae was 0.4-0.8 days longer than that in the water control ( $F = 5.01$ ;  $df = 3, 84$ ;  $P = 0.003$ ). The overall developmental time from egg to adult emergence was 26.5-27.9 days, 3.2-4.6 days longer than 23.3 days in the water control ( $F = 104.53$ ;  $df = 3, 84$ ;  $P = 0.0001$ ) (Table 1). When first instars were treated, there were no significant effects on the subsequent development of the first instar ( $F$

$= 1.63$ ;  $df = 3, 100$ ;  $P = 0.1868$ ) and the second instar ( $F = 0.78$ ;  $df = 3, 100$ ;  $P = 0.51$ ) (Fig. 2B). However, the developmental times of treated first instars were delayed at the third instar and pupal stages at all of the three concentrations. The developmental time of the third instar was 1.6-2.1 days longer than that in the water control ( $F = 32.02$ ;  $df = 3, 100$ ;  $P = 0.0001$ ), and that of the pupae was 2.6-3.0 days longer than that in the water control ( $F = 3.13$ ;  $df = 3, 92$ ;  $P = 0.0295$ ). The overall developmental time from first instar to adult emergence was 21.7-22.3 days compared with 19.3 days in the water control ( $F = 24.80$ ;  $df = 3, 92$ ;  $P = 0.0001$ ) (Table 1). When second instar was treated, the developmental time of the second instar was prolonged 0.3-0.5 days ( $F = 2.86$ ;  $df = 3, 108$ ;  $P = 0.0404$ ), and that of the third instar was prolonged 1.4-2.0 days ( $F = 27.48$ ;  $df = 3, 106$ ;  $P = 0.0001$ ) compared to that in the water control (Fig. 2C). The developmental time of pupae was not significantly prolonged among the four treatments ( $F = 1.87$ ;  $df = 3, 100$ ;  $P = 0.1393$ ). The overall developmental time from second instar to adult emergence was 19.8-20.5 days compared with 17.7 days in the water control ( $F = 28.43$ ;  $df = 3,$

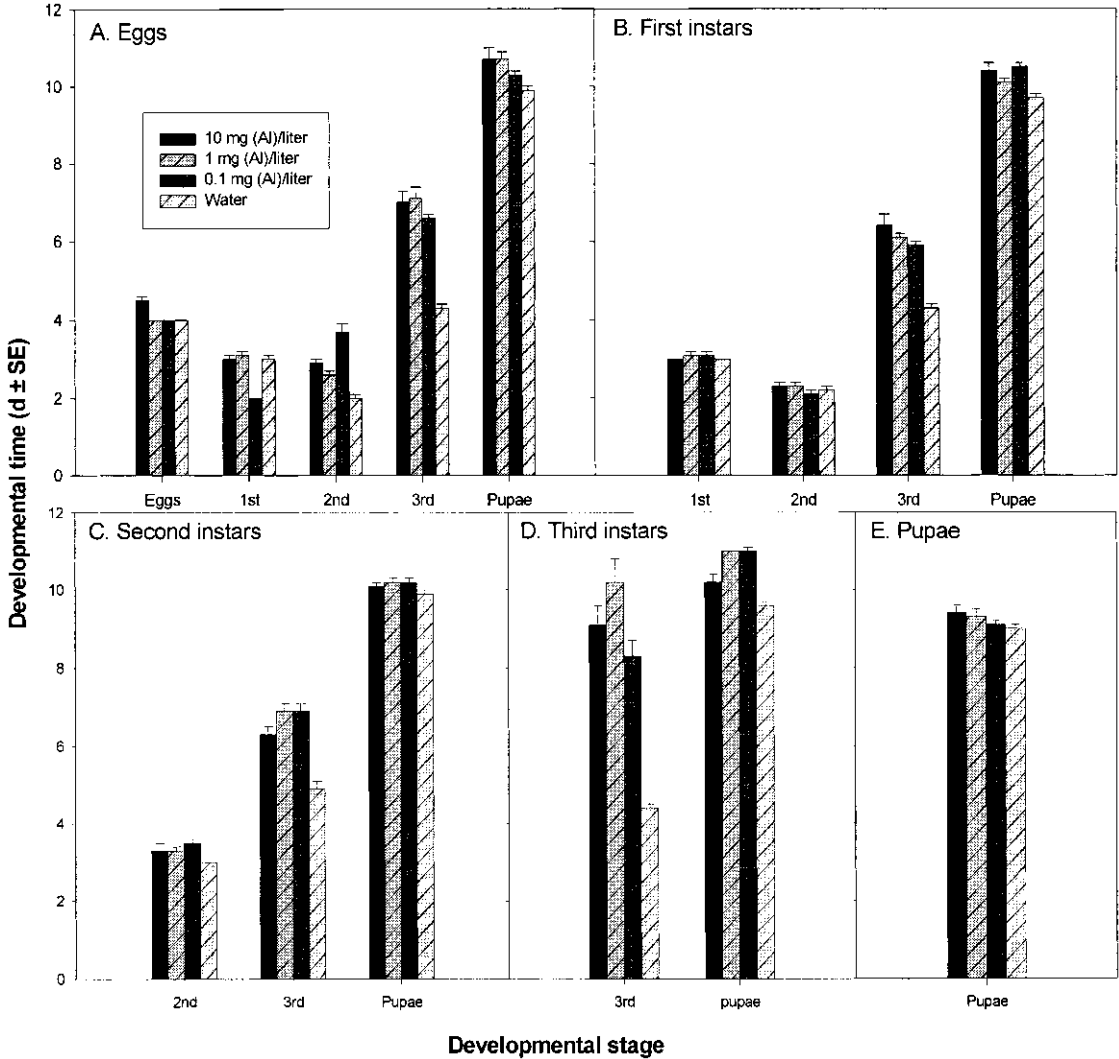


Fig. 2. Developmental time of eggs, larvae and pupae of *Chrysoperla rufilabris* after treatment with three concentrations of fenoxycarb at different developmental stages.

100;  $P = 0.0001$ ) (Table 1). When third instars were treated, developmental time was delayed at the third instar and pupal stage (Fig. 2D). The developmental time of the third instar was 3.9-4.7 days longer than that in the water control ( $F = 67.20$ ;  $df = 3, 60$ ;  $P = 0.0001$ ), and that of the pupae was 0.6-1.4 days longer than that in the water control ( $F = 8.28$ ;  $df = 3, 32$ ;  $P = 0.0001$ ). The overall developmental time from third instar to adult emergence was 18.6-20.0 days compared with 14.0 days in the water control ( $F = 96.37$ ;  $df = 3, 32$ ;  $P = 0.0001$ ) (Table 1). When pupae were treated, the developmental times of pupae did not differ significantly among the four treatments ( $F = 1.83$ ;  $df = 3, 100$ ;  $P = 0.1469$ ) (Fig. 2E, Table 1).

## DISCUSSION

Our results indicated that application of fenoxycarb to eggs and larvae of *C. rufilabris* resulted in decreased survival rates and prolonged development times when treated at different immature stages and at different concentrations. Fenoxycarb showed significant ovicidal effect on *C. rufilabris* eggs, with 66.7-80.0% survival rates, depending on the larval stages treated and the concentrations used. Generally, the higher concentrations exhibited greater effects on the larval stages treated and the subsequent stages of the larvae. Similar effect has been reported on *C. carnea* by Bigler and Waldburger (1994) and Celli

TABLE 1. EFFECT OF FENOXYCARB ON THE DEVELOPMENT OF *CHRYSOPERLA RUFILABRIS* WHEN TREATED AT DIFFERENT DEVELOPMENTAL STAGES.

Concentration, Mg (AI)/liter	Stage treated and developmental time ( $d \pm SE$ ) <sup>a</sup>				
	Eggs	1st instars	2nd instars	3rd instars	Pupae
10	27.9 $\pm$ 0.3a	22.3 $\pm$ 0.3a	19.8 $\pm$ 0.4b	20.0 $\pm$ 0.8b	9.4 $\pm$ 0.2
1	27.2 $\pm$ 0.3b	21.7 $\pm$ 0.5a	20.5 $\pm$ 0.2a	21.5 $\pm$ 2.5a	9.3 $\pm$ 0.2
0.1	26.5 $\pm$ 0.2c	21.6 $\pm$ 0.2a	20.5 $\pm$ 0.2c	18.6 $\pm$ 0.6c	9.1 $\pm$ 0.1
Water	23.3 $\pm$ 0.2d	19.3 $\pm$ 0.1b	17.7 $\pm$ 2.d	14.0 $\pm$ 0.1d	9.0 $\pm$ 0.1
<i>F</i>	104.53	24.81	28.43	96.37	1.83
<i>P</i>	0.0001	0.0001	0.0001	0.0001	0.1469
df	3, 84	3, 92	3, 100	3, 32	3, 100

<sup>a</sup>Means in the same column followed by the same letters do not differ significantly at  $P = 0.05$  (LSD, SAS Institute 1996).

et al. (1997). We do not know why fenoxycarb shows ovicidal effects on some insects (including *Chrysoperla*), but not on others. Charmillot et al. (1985) observed that fenoxycarb showed more severe effects on the eggs laid singly than those laid in egg masses. They suggested that the difference may be due to a lack of contact or a reduction of contact between the eggs in masses and the treated leaf surfaces. This explanation is consistent with the poor ovicidal performance of fenoxycarb on some insects with egg masses, but exhibiting severe ovicidal effect on *Chrysoperla* spp. that lay their eggs singly.

We observed that among the immature stages, the third instar was the most susceptible and vulnerable stage with the highest mortality and longest developmental delay regardless whether the egg, first, second, or third instar was treated. We do not know why the third instar is the most susceptible stage. Generally, the juvenile hormone (JH) titers gradually decrease as the larvae approach pupation, in this case, the third instar larvae of *C. rufilabris*. One possible explanation might be that application of fenoxycarb to third instar larvae overdosed the JH level in the larvae, and the JHA fenoxycarb could not be metabolized before the metamorphosis. As a result, metamorphosis (pupation) is disturbed or blocked. We observed that some third instars that survived had difficulty spinning cocoons. Some did not have silk to make the cocoon, whereas others produced isolated silk threads, but could not make a complete cocoon. Some larvae managed to pupate even in an incomplete cocoon. All those that did not make cocoons or did not have a complete cocoon died at the pupal stage or as pharate adults. Compared with the third instars, the first and the second instars have relatively high levels of JH, and have sufficient time and ability to metabolize the added juvenile analog.

Fenoxycarb not only causes high mortalities and prolonged developmental times on *Chrysoperla* spp., but also inhibits egg production of the adult *C. carnea* when second or third instars were

treated (Celli et al. 1997). As reported in the literature (i.e., Grenier & Plantevin 1990; Grenier & Grenier 1993), fenoxycarb is toxic to other predators and parasitoids, such as *Anthocoris*, *Chilocorus*, and some species in Tachinidae, Braconidae and Aphelinidae, as well as many other beneficial insects, such as silkworms and bees. Our results indicate that fenoxycarb is nonselective to *Chrysoperla* spp. and cannot be used where these predators are dominant. Although data obtained from laboratory toxicity studies have been sufficient to decide upon the use of insecticides in IPM (in cases where mortality was low in laboratory experiments) (Barrett et al. 1994), semi-field and field studies are still needed. More research on the effects of fenoxycarb on lacewings, and other predators and parasitoids under different agroecosystems is also needed to elucidate how to use IGRs in IPM programs.

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