# Compatibility of Insect Growth Regulators with *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for Whitefly (Homoptera: Aleyrodidae) Control on Poinsettias

I. Laboratory Assays

Mark S. Hoddle,\* R. G. Van Driesche,† S. M. Lyon,† and J. P. Sanderson‡

\* Department of Entomology, University of California, Riverside, California 92521; †Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003; and ‡Department of Entomology, Cornell University, Ithaca, New York 14583

Received February 18, 2000; accepted August 12, 2000; published online December 12, 2000

The compatibility of five insect growth regulators (IGRs), buprofezin, pyriproxyfen, fenoxycarb, pymetrozine, and kinoprene, were tested in the laboratory for compatibility with the whitefly parasitoid Eretmocerus eremicus Rose and Zolnerowich (Hymenoptera: Aphelinidae). The survivorship of adult parasitoids foraging on poinsettia leaves with residues 6, 24, and 96 h of age was determined. The toxicity of Bemisia argentifolii Bellows and Perring (Homoptera: Aleyrodidae) patches treated with IGRs presented to female parasitoids 24 and 96 h posttreatment was quantified. Survivorship of immature E. eremicus developing within **B.** argentifolii nymphs was determined by treating whitefly nymphs with IGRs 5 and 13 days postoviposition by female parasitoids. Finally, behavioral observations of female parasitoids foraging on IGR-treated and untreated B. argentifolii patches presented simultaneously were quantified to determine whether IGR residues had a repellant effect toward E. eremicus. Averaging ranks for IGRs based on their compatibility with E. eremicus and their ability to kill B. argentifolii nymphs produced the following parasitoid compatibility order: buprofezin > fenoxycarb > pymetrozine = pyriproxyfen > kinoprene. Further work in greenhouses assessing the efficacy of buprofezin with E. eremicus for B. argentifolii control on poinsettias is recommended. • 2000 Academic Press

Key Words: Eretmocerus eremicus; Bemisia argentifolii; poinsettia; greenhouse; insect growth regulator; buprofezin; pyriproxyfen; fenoxycarb; pymetrozine; kinoprene; natural enemy compatibility; integrated pest management.

# **INTRODUCTION**

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (=*Bemisia tabaci* [Gennadius] strain B)

(Homoptera: Aleyrodidae), is a foliar pest of greenhouse poinsettias (Euphorbia pulcherrima Willd. ex. Koltz.) that can be effectively controlled with the parasitoid Eretmocerus eremicus Rose and Zolnerowich (Hymenoptera: Aphelinidae) (Hoddle et al., 1998a, 1999). Results of trials in commercial greenhouses with cooperating growers have demonstrated that E. eremicus can control B. argentifolii on poinsettia stock plants grown over summer for cuttings (Hoddle and Van Driesche, 1999a) and on poinsettias grown for color and sale at Christmas (Hoddle and Van Driesche, 1999b). E. eremicus can also control another foliar pest of poinsettia, the greenhouse whitefly, Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae), when whitefly populations consist solely of T. vaporariorum or are sympatric with B. argentifolii (Van Driesche et al., 1999). Furthermore, unsupervised growers have demonstrated the ability to order and deploy E. eremicus in commercial greenhouses holding poinsettias infested with whiteflies, and these growers successfully managed parasitoid releases to maintain pest densities at nondamaging levels (Van Driesche et al., 1999).

A major constraint to commercial widespread adoption of biologically based whitefly management in greenhouses using whitefly parasitoids is cost (Hoddle and Van Driesche, 1996, Hoddle and Van Driesche, 1999b; Van Driesche *et al.*, 1999). In comparison to applications of insecticides (i.e., imidacloprid), whitefly control using *E. eremicus* as the major component for whitefly suppression is 17–30 times more expensive (Hoddle and Van Driesche, 1996, a,b; Van Driesche *et al.*, 1999). The use of *E. eremicus* can become cost effective if commercial suppliers minimize purchase costs by making parasitoid production as inexpensive and efficient as possible, and increased demand for *E. eremicus* by growers promotes increased supply and



lower prices. The combined effect of these two factors has already reduced the cost of *E. eremicus* by 89% from the time that this parasitoid was first marketed commercially in the United States in 1994 (Hoddle and Van Driesche, 1999a). Finally, cost can be lowered by development of release programs that minimize the number of parasitoids required for effective *B. argentifolii* control.

Reduction of the number of parasitoids that are purchased and released weekly could be achieved by manipulating release rates to promote high levels of parasitism early in the cropping season so that releases of parasitoids later in the cropping cycle can be reduced or eliminated. Whitefly control would then result from the progeny of previously released parasitoids (Hoddle et al., 1999). High levels of parasitoid reproduction during the cropping season can augment weekly releases to significantly reduce pest densities. However, this strategy of reliance on parasitoid reproduction may be too unpredictable to produce marketable plants by a given deadline (Hoddle et al., 1997). Alternatively, parasitoid release rates could be minimized if reduced whitefly mortality from parasitoid activity could be compensated for with another control practice that growers are familiar with using. An additional mortality agent to supplement parasitoid activity could be the incorporation of a parasitoid-compatible insecticide such as an insect growth regulator (IGR) into an integrated pest management program for *B. argentifolii* control on poinsettias.

Insect growth regulators are efficacious whitefly control materials and have been used extensively in the management of *Bemisia* spp. and *T. vaporariorum* in greenhouses and outdoor agricultural crops (Dittrich *et al.*, 1990; Boukadida and Michelakis, 1994; Horowitz and Ishaaya, 1994). Several IGRs have been shown to be highly compatible with certain whitefly parasitoids, as they interfere minimally with their developmental and reproductive biology and the foraging behavior of females is not adversely affected (Gerling and Sinai, 1994; Liu and Stansly, 1997; Jones *et al.*, 1998). Consequently, IGRs appear to be promising sources of mortality to complement *E. eremicus* activity in greenhouses for whitefly control.

A consistent trend observed in *E. eremicus* trials in commercial greenhouses is a mid-season increase in whitefly numbers that continues to climb slowly until plants are harvested. A fixed weekly parasitoid release rate results, as plants grow, in an increasing dilution of the number of parasitoids searching per leaf. Consequently, greater numbers of whitefly nymphs escape discovery, their mortality rates decline, and whitefly numbers subsequently increase (Hoddle *et al.*, 1998a,b, 1999; Hoddle and Van Driesche, 1999a). Whitefly population growth during the cropping season is faster when parasitoid release rates are low because parasitoid dilution due to plant growth is more rapid than

that in greenhouses treated concurrently with higher release rates of parasitoids (Hoddle et al., 1998a,b, 1999). Consequently, a whitefly control strategy that utilizes reduced parasitoid release rates will have to incorporate a density-independent mortality factor to retard the tendency for whitefly densities to increase over the growing season. We hypothesize that halting parasitoid releases mid-season and applying a parasitoid-compatible IGR to reduce whitefly densities could significantly reduce whitefly population growth in biological control greenhouses treated with low parasitoid release rates. Low release rates of *E. eremicus* would continue after spraying with the assumption that parasitoids could maintain whitefly levels at the lowered densities achieved with the IGR until crop harvest. Before this hypothesis could be tested in greenhouses we needed to identify IGRs that would be compatible with *E. eremicus* for whitefly control on poinsettias.

The work presented here investigated five IGRs (buprofezin, pyriproxyfen, fenoxycarb, pymetrozine, and kinoprene) for compatibility with *E. eremicus.* These IGRs were chosen because of their known ability to kill whiteflies. Specifically, we sought to identify compatible IGRs by (1) determining the effect that IGR residues on poinsettia leaves had on survivorship of foraging *E. eremicus* adults, (2) ascertaining the effect that IGRs had on *E. eremicus* survival when females used IGR-treated *B. argentifolii* nymphs for host feeding and oviposition, (3) assessing the effect that IGR applications had on survivorship of immature E. eremicus developing within *B. argentifolii* nymphs, and (4) establishing whether IGR residues adversely affected the foraging behavior of female *E. eremicus* by making leaves repellant to parasitoids. By addressing these four issues in the laboratory we sought to identify from among five candidate IGRs those that would be compatible with *E. eremicus* for *B. argentifolii* control on poinsettias for further evaluation in greenhouses.

#### **MATERIALS AND METHODS**

# Preparation of IGRs on Whole Plants for Presentation to E. eremicus

Five IGRs plus a water control were applied separately to single-stem poinsettia plants (cv. 'Freedom Red') that bore 8–12 leaves. Experimental plants were grown and maintained in a plastic-covered greenhouse. Plants were treated by being dipped and gently agitated for 30 s in 10-L buckets containing solutions of test materials. IGR residues on plants were allowed to dry and age in a plastic-covered greenhouse before use in experiments. IGR concentrations were either based on the labels if the test compound was registered for use on greenhouse ornamentals or based on the recommendations of the manufacturing company's technical representative if the IGR was not registered for greenhouse use. If the application rate was presented as a recommended range, the maximum recommendation was tested. The materials used, IGR trade names, manufacturers, [amount of measured product per liter of water], and formulations were buprofezin (Applaud, AgrEvo USA Co. [now Aventis], Wilmington DE, [285 mg/L],WP 70% ai), pyriproxyfen (Knack, Valent USA Co., Walnut Creek CA, [1.45 ml/L], EC 11% ai), fenoxy-carb (Precision, CIBA-Geigy Corp. [now Novartis], Greensboro NC, [299 mg/L], WP 25% ai), pymetrozine (Fulfill, CIBA-Geigy Corp. [now Novartis], Greensboro NC, [373 mg/L], WP 25% ai), and kinoprene (Enstar II, Sandoz Agro Inc., [now Novartis], Des Plaines IL, [1.33 ml/L], AC 65% ai).

#### Source of Parasitoids and Whiteflies for Experiments

*E. eremicus* was supplied by Beneficial Insectaries, Oak Run, California as loose parasitized *T. vaporariorum* nymphs packaged in sawdust. Each shipment was divided among three clear plastic emergence cages  $(30 \times 30 \times 30 \text{ cm})$  and parasitoids were left to emerge at  $25.2 \pm 1.4^{\circ}$ C under long days of 14:10 (L:D) h. Each cage was furnished with yellow paper finely streaked with honey as a carbohydrate source for parasitoids. Shipments of parasitoids were ordered to be received within 24–36 h of an experiment, and all adult parasitoids were 24 h of age or younger when aspirated from emergence cages and used for experiments.

A *B. argentifolii* colony was maintained in an insectary room at  $25.7 \pm 0.8$ °C under long days of 14:10 (L:D) h on poinsettias (cv. 'Freedom Red') at the University of Massachusetts, Amherst. Adult whiteflies were removed from this colony and used for experiments when necessary.

# *Caging E. eremicus and B. argentifolii on Poinsettia Leaves*

For experiments that required caging either whiteflies or parasitoids on leaves, one mature leaf was randomly selected on each of 20 plants per treatment, and one glass cell (height = 15 mm; diameter = 28 mm) was attached to the underside of selected leaves with a ring of Duco Stik-Tak (Devcon Consumer Products, Des Plaines, IL). The upper side of the glass cell was covered with polyester mesh screening with a 95- $\mu$ m opening size. A 5-mm-diameter opening on the side of the glass cell provided access to the cell interior once cells were attached to leaves with Duco Stik-Tak. This aperture could be closed with a cork.

Parasitoids were placed in glass cells on leaves by aspirating adults into disposable flint glass Pasteur pipettes (length = 15 cm; Fisher Scientific, Pittsburgh, PA) from emergence cages. Parasitoids were prevented from walking into the main pipette chamber by a wad of polyester mesh placed in the bore of the pipette approximately 2-3 cm from the shortened pipette tip. Tips of pipettes were placed inside glass cells through the side opening and gently tapped until all parasitoids entered the experimental arena. The pipette was then removed and the aperture closed with a cork.

To create cohorts of whitefly nymphs for host feeding and parasitism studies, four mating pairs of adult whiteflies were aspirated from the *B. argentifolii* colony into glass pipettes similar to those used for parasitoids. Adult whiteflies were released into glass cells in the same manner as parasitoids and were left to oviposit for 2 days. After this time cages were removed and adults were aspirated from leaves. Whitefly nymphs developed on poinsettia plants in the laboratory at  $25.2 \pm 1.4$ °C under long days 14:10 (L:D) h until the appropriate immature stages had been reached (inspected daily and estimated by eye) for use in experiments as determined by daily visual observation.

# Survivorship of Adult E. eremicus Foraging on IGR-Treated Poinsettia Leaves without B. argentifolii Nymphs

For each IGR tested and the water control, 20 singlestem poinsettia plants were dipped in one of the five IGR solutions or water. Following dipping, plants were returned to the greenhouse and residues were allowed to dry. At 6, 24, and 96 h posttreatment, plants were removed from the greenhouse and returned to the laboratory and residues were evaluated for toxicity to E. eremicus. To determine the toxicity of aged IGR residues on poinsettia leaves lacking *B. argentifolii*, the survivorship of a total of five adult *E. eremicus* (males and females combined) placed inside glass cells with pipette aspirators was determined after 24 h using a dissecting microscope. The mean number of surviving adult parasitoids exposed to dried IGR residues per treatment in each age category and water controls was compared using ANOVA in SAS (SAS, 1989). There was no carbohydrate source for parasitoids inside glass cells.

# Survivorship of E. eremicus Foraging on IGR-Treated Poinsettia Leaves with B. argentifolii Nymphs

For each IGR treatment and the water control, cohorts of *B. argentifolii* nymphs for host feeding or oviposition by female *E. eremicus* were established on one randomly selected poinsettia leaf on each of 40 plants as described above. Following nymphal eclosion from eggs, leaves bearing cohorts of *B. argentifolli* nymphs were treated with IGRs when immature whiteflies were first and second instars, the preferred stages for host feeding (Headrick *et al.*, 1995, 1996). Treated plants bearing cohorts of *B. argentifolii* nymphs were returned to the greenhouse. Either 24 or 96 h after IGR treatments, plants were taken from the greenhouse and returned to the laboratory where glass cells were attached to leaves with Duco Stik-Tak to enclose cohorts of *B. argentifolii* nymphs. Subsequently, five female *E. eremicus* were placed in each glass cell and allowed to forage on treated nymphs for 24 h. Survivorship of foraging females was recorded on each of 20 plants for each IGR treatment aged for either 24 or 96 h in the greenhouse after IGR application. The mean number of surviving adult parasitoids exposed to IGR-treated *B. argentifolii* nymphs for each treatment in each age category was compared using ANOVA in SAS (SAS, 1989).

# Survivorship of Immature E. eremicus within B. argentifolii Nymphs Following IGR Applications

For each IGR treatment and the water control, cohorts of *B. argentifolii* nymphs were established on one randomly selected poinsettia leaf on each of 40 plants as described above and presented to female parasitoids. Following nymphal eclosion from eggs, cohorts of *B. argentifolli* nymphs were recaged with glass cells when immatures became second and third instars (the whitefly lifestages used by *E. eremicus* for oviposition [Headrick *et al.*, 1995, 1996]). All whitefly cohorts had a minimum of 60 nymphs, a number considered adequate to accommodate both the host feeding and the oviposition needs of female parasitoids.

Once whitefly nymphs reached the appropriate stages for parasitism, one female parasitoid was aspirated from an emergence cage and released into the glass cell enclosing whitefly nymphs. Each female was left to forage for 24 h before being removed. The mating status of each female was undetermined, but given the existence of males in emergence cages and observed matings in cages, it is highly probable that mated and unmated females were assigned randomly to treatments following removal from emergence cages. Plants were maintained in the laboratory at 25.2°C until parasitoid larvae were estimated to have reached designated ages for treatment with IGRs.

At 25.2°C, parasitoid larvae were estimated to be first instar larvae 5 days after oviposition had occurred (Foltyn and Gerling, 1985). At this time, 20 plants for each IGR treatment or the water control were individually dipped in a solution of test material to determine the susceptibility of young *E. eremicus* larvae to treatments. Following treatment, plants were maintained in the laboratory. Following a 7-day period, plants were examined daily for parasitoid emergence and the number of whiteflies bearing parasitoid exit holes was recorded for each leaf bearing whitefly cohorts on plants assigned to each treatment.

At 25.2°C, parasitoid larvae were estimated to be late third instar larvae or young pupae 13 days postoviposition (Foltyn and Gerling, 1985). At this time, the remaining 20 plants for each IGR treatment or the water control were individually dipped in test solutions to determine the susceptibility of *E. eremicus* larvae in the late stages of development to treatments. Following IGR and water treatments, plants were maintained in the laboratory. Plants were examined daily posttreatment for parasitoid emergence and the number of whiteflies bearing parasitoid exit holes was recorded for each leaf bearing whitefly cohorts on plants assigned to each treatment. The mean number of successfully emerged adult parasitoids exposed to IGR-treated *B. argentifolii* nymphs for each treatment in each age category was compared using ANOVA in SAS (SAS, 1989). The proportion of whitefly nymphs killed in each cohort for each IGR and water treatment was recorded and square root arc-sine transformed, and mortality rates were compared using ANOVA in SAS (SAS, 1989).

# Behavioral Effects of IGR Residues on Foraging E. eremicus Females

The behavior of female *E. eremicus* foraging on poinsettia leaves with B. argentifolii nymphs and IGR residues was determined in the laboratory. Detached poinsettia leaves bearing two cohorts of second and third instar B. argentifolii nymphs (one cohort each on either side of the leaf midrib on the abaxial surface) had one side dipped laterally up to, but not including the midrib, in an IGR solution. The other side of the leaf was dipped in water, which acted as a control for a dipping effect. Only one leaf side of water controls was dipped. Leaf sides were randomly assigned to treatments before dipping. Residues on detached leaves were left to dry in the laboratory for 1.5 h before presentation to female parasitoids. Kinoprene and pyriproxifen residues had to be aged for 48 h before presentation to parasitoids because of extremely high rates of premature abandonment and behavioral assays were repeated with residues 48 h of age (see Results).

Individual female *E. eremicus* ( $\leq$ 24 h old) were removed from emergence cages and individually placed on untreated midribs (abaxial side facing up) of unenclosed experimental leaves with a camel hair brush. Females were observed with a dissecting microscope, the experimental leaf was illuminated with a fiber optic light source, and behavioral events were recorded using a laptop computer with an event recorder program (Eventrecorder, CV Amsterdam, The Netherlands). Four positional events were recorded with the event recorder: (1) female foraging on non-IGR-treated surface and off the whitefly patch, (2) female foraging on a non-IGR-treated surface and on the whitefly patch, (3) female foraging on IGR-treated surface and off the treated whitefly patch, and (4) female foraging on an IGR-treated surface and on the treated whitefly patch. Observations of foraging females were made for 5 min. If females abandoned leaves prior to this 5-min test period, premature abandonment was recorded and

another replicate initiated. This was repeated until 30 individual females were each observed to complete 5-min foraging bouts for each IGR and water treatment.

The abilities of 1.5-h-old IGR residues to induce premature leaf abandonment were compared across treatments using the  $\chi^2$  test. Analyses of time allocation were made only for parasitoids completing the 5-min foraging bout. Duration of time (total time in seconds) in each of the four positional events was square root transformed and tested for conformity to a normal distribution using a Wilkes–Shapiro Test (SAS, 1989). Time in a positional event was compared among IGRs with ANOVA and treatment means were separated with Sheffe's test at the 0.05 level of significance (SAS, 1989).

#### RESULTS

# Survivorship of Adult E. eremicus Foraging on Treated Poinsettia Leaves without B. argentifolii Nymphs

Significant differences in parasitoid survivorship were observed among IGRs and the water controls that were aged for 6 h in the greenhouse before caging of *E. eremicus* (F = 14.03, df = 5, 114, P < 0.0005). Highest average parasitoid survivorship per glass cell after the 24-h exposure period was observed for the water controls, and survivorship was lowest for kinoprene (Fig. 1A).

Residues aged for 24 h in the greenhouse exhibited significant differences in compatibility with adult *E. eremicus* caged for 24 h on treated poinsettia leaves (F = 5.86, df = 5, 114, P < 0.0005). Parasitoids exhibited highest survivorship on water and on fenoxycarband pymetrozine-treated leaves. Survivorship was lowest on kinoprene-treated leaves (Fig. 1B).

There were no significant differences among IGR residues and water controls that had been aged for 96 h in the greenhouse before caging of adult *E. eremicus* for a 24-h period (F = 1.37, df = 5, 114, P = 0.24) (Fig. 1C).

# Survivorship of E. eremicus Foraging on IGR-Treated Poinsettia Leaves with B. argentifolii Nymphs

Survivorship of caged female *E. eremicus* foraging on poinsettia leaves with *B. argentifolii* nymphs that had been treated with IGRs or water and then aged for 24 h in a greenhouse differed significantly across treatments (F = 6.93, df = 5, 109, P < 0.0005). Female survivorship was highest on water and on buprofezinand fenoxycarb-treated leaves, intermediate on leaves with pyriproxyfen or pymetrozine residues, and lowest on kinoprene-treated leaves (Fig. 2A).

Significant differences were observed in parasitoid survivorship levels among IGR residues and water con-

trols after residues were aged in the greenhouse for 96 h before female parasitoids were caged on treated *B. argentifolii* patches for 24 h (F = 15.52, df = 5, 158 P < 0.0005). Parasitoid survivorship was highest on water and on pymetrozine- and buprofezin-treated leaves, intermediate on pyriproxyfen- and fenoxycarb-treated leaves, and lowest on leaves with kinoprene residues (Fig. 2B).

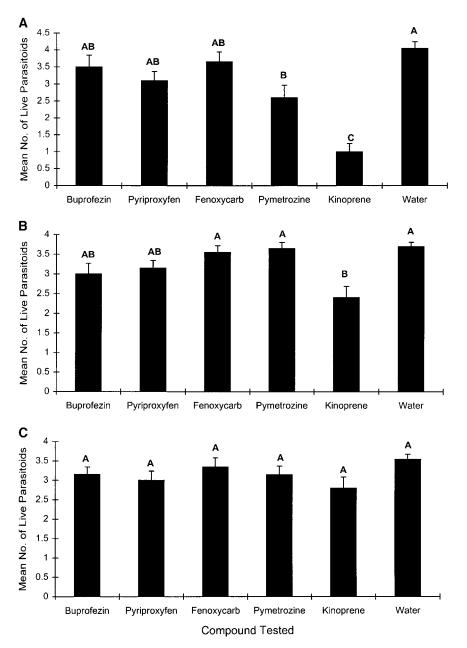
# Survivorship of Immature E. eremicus within B. argentifolii Nymphs Following IGR Applications

Significant differences in the mean number of *B*. argentifolii nymphs from which parasitoids successfully emerged were observed among IGR treatments and the water controls when treatments were applied 5 days post parasitoid exposure (i.e., when young E. er*emicus* larvae were treated with IGRs) (F = 13.18, df =5, 98, P < 0.0005). Young parasitoid larvae managed to survive and emerge at varying levels in all IGR treatments. Successful parasitoid emergence was greatest for water controls and lowest for kinoprene-treated leaves (Fig. 3A). The mean proportion of *B. argentifolii* nymphs killed by IGRs following dipping 5 days after exposure to parasitoids varied significantly between treatments (F = 62.29, df = 5, 98, P < 0.0005). Whitefly mortality was greatest for buprofezin, pyriproxyfen, and kinoprene treatments, and lowest for leaves dipped in water. Mortality of *B. argentifolii* nymphs on water-treated plants indicates the level of death caused by host feeding, unsuccessful parasitism, and other mortality not related to parasitoid activity or IGR residues (Fig. 3B).

*E. eremicus* larvae in the late stages of development (13 days postoviposition) were not affected by IGR applications and no significant differences were detected in the mean numbers of parasitoids successfully emerging among treatments (F = 0.87, df = 5, 99, P =0.50) (Fig. 3C). Whitefly mortality differed significantly among treatments following IGR treatments 13 days after parasitoid oviposition (F = 11.48, df = 5, 99, P <0.0005). Whitefly mortality was highest on buprofezinand pyriproxyfen-treated plants and lowest on watertreated leaves (Fig. 3D). Whitefly mortality was consistently higher across all treatments on plants that were treated 5 days post parasitoid exposure than on plants treated 13 days post parasitoid exposure (Figs. 3B and 3D). This result suggests that young whitefly nymphs are more susceptible to IGR treatments than older nymphs.

# Behavioral Effects of IGR Residues on Foraging E. eremicus Females

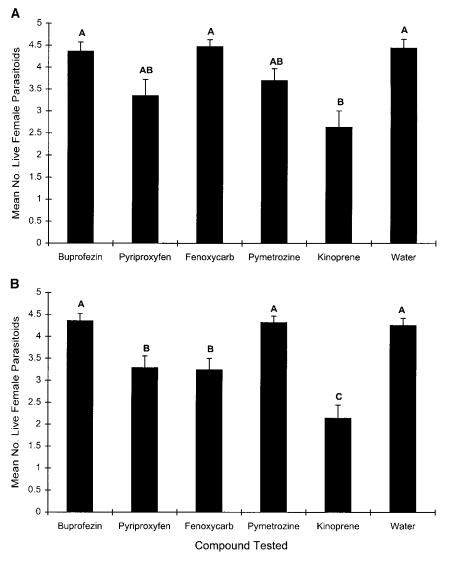
Significant differences in leaf abandonment by parasitoids before the completion of a 5-min foraging bout were observed across IGR-treated leaves on which residues were 1.5 h of age ( $\chi^2 = 30.23$ , df = 5, P < 0.005).



**FIG. 1.** The mean number of surviving adult *Eretmocerus eremicus* after being caged in glass cells for 24 h on treated poinsettia leaves lacking *Bemisia argentifolii* nymphs once residues had been aged in a greenhouse for (A) 6 h, (B) 24 h, and (C) 96 h. Columns with the same letters are not significantly different at the 0.05 level (Tukey's Studentized range test).

Repeating the  $\chi^2$  test with the removal of kinoprene and pyriproxyfen (both liquid IGRs) from the data set failed to reveal significant differences in frequency of parasitoid departures from treated leaves across treatments ( $\chi^2 = 3.10$ , df = 3, P = 0.38). Kinoprene, pyriproxyfen, and a water control were reexamined for repellency and likelihood of leaf abandonment prior to the allocated 5 min of foraging time after residues were aged for 48 h. No significant differences in abandonment were observed on leaves treated with kinoprene, pyriproxyfen, or water after residues had aged for a 48-h period ( $\chi^2 = 0.23$ , df = 2, P = 0.89). Consequently, subsequent behavioral studies with kinoprene and pyriproxyfen were conducted with residues aged for 48 h for comparison with buprofezin, fenoxycarb, and pymetrozine residues that had been dried for 1.5 h.

No significant differences were observed across treatments for time spent by parasitoids on *B. argentifolii* patches on untreated leaf sides (F = 1.26, df = 5, P = 0.29) (Table 1). Female *E. eremicus* spent significantly less time on *B. argentifolii* patches on leaf sides treated with kinoprene and pyriproxyfen



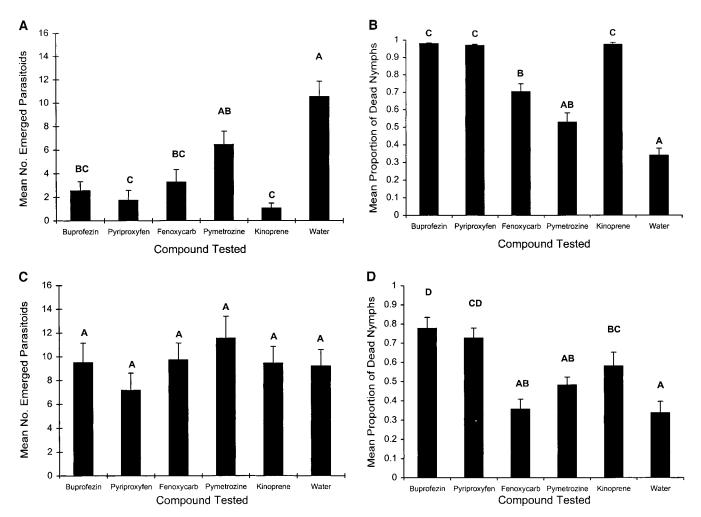
**FIG. 2.** The mean number of surviving female *Eretmocerus eremicus* after being caged in glass cells for 24 h on treated poinsettia leaves with first and second instar *Bemisia argentifolii* nymphs (A) 24 h and (B) 96 h post IGR and water treatment. Columns with the same letters are not significantly different at the 0.05 level (Tukey's Studentized range test).

(F = 3.16, df = 4, P < 0.005). Pyriproxyfen-treated whitefly nymphs appeared to deter female *E. eremicus* from oviposition and host-feeding behaviors, as no individuals were observed for recordable periods on *B. argentifolii* patches treated with this IGR (Table 1). Consequently, on pyriproxyfen-treated leaves, females spent significantly more time walking on untreated leaf sides and off whitefly patches (F = 3.70, df = 5, P < 0.05). A significant increase in time spent on untreated leaf sides and off whitefly patches by parasitoids on kinoprene-treated leaves was not observed (Table 1). Female parasitoids spent equal amounts of time on treated leaf surfaces and off whitefly patches (F = 1.71, df = 5, P = 0.14), suggesting that dried residues alone in the age classes

presented were not sufficient to repel parasitoids from treated leaves (Table 1).

### DISCUSSION

The IGRs tested (buprofezin, pyriproxyfen, fenoxycarb, pymetrozine, and kinoprene) in this study showed differences in their compatibility with *E. eremicus* with respect to toxicity on poinsettia leaves that lacked *B. argentifolii* nymphs when residues were dried and aged in a greenhouse. IGRs most compatible with *E. eremicus* (i.e., mortality rates were not significantly different from water treatments) 6 h postapplication were buprofezin, pyriproxyfen, and fenoxycarb. Kinoprene-treated leaves caused highest levels of



**FIG. 3.** (A) The mean number of *Eretmocerus eremicus* successfully emerging from *Bemisia argentifolii* nymphs treated with IGRs or water 5 days post parasitoid oviposition. (B) The mean proportion of dead *B. argentifolii* nymphs following treatment with IGRs or water 5 days post parasitoid oviposition. (C) The mean number of *E. eremicus* successfully emerging from *B. argentifolii* nymphs treated with IGRs or water 13 days post parasitoid oviposition. (D) The mean proportion of dead *B. argentifolii* nymphs following treatment with IGRs or water 13 days post parasitoid oviposition. (D) The mean proportion of dead *B. argentifolii* nymphs following treatment with IGRs or water 13 days post parasitoid oviposition. Columns with the same letters are not significantly different at the 0.05 level (Tukey's Studentized range test).

parasitoid mortality 6 h posttreatment. Following an aging period of 24 h in a greenhouse, kinoprene was still the most toxic IGR to *E. eremicus.* After aging for 96 h in a greenhouse, IGR residues did not adversely affect parasitoid survivorship in comparison to water controls. Results from other studies indicate that buprofezin residues on leaves have negligible impact on foraging adult *Eretmocerus* sp. (Jones *et al.*, 1995, 1998). Similarly, pyriproxyfen leaf residues appear to have little effect on survivorship of adult whitefly parasitoids in the genus *Encarsia* (Hymenoptera: Aphelinidae) (Liu and Stansly, 1997), and we observed similar effects with *E. eremicus*.

Lethality of IGRs to *E. eremicus* differed when *B. argentifolii* nymphs treated with IGRs were present on poinsettia leaves for female parasitoids to use for either host feeding or oviposition. Buprofezin and

fenoxycarb were the most compatible with E. eremicus after 24 h posttreatment of whitefly nymphs and kinoprene caused the highest levels of parasitoid mortality at this time. After an additional 48 h in the greenhouse (i.e., residues were now 96 h of age) buprofezin and pymetrozine treatments had parasitoid survivorship levels similar to those of the water controls. The toxicity of fenoxycarb to E. eremicus had significantly increased when this IGR was aged for 96 h in comparison to parasitoid mortality rates observed after 24 h. Highest levels of parasitoid mortality were observed on kinoprene-treated leaves after 96 h. Residues aged in the greenhouse for 96 h on poinsettia leaves without B. argentifolii nymphs had similar parasitoid survivorship rates and were not significantly different from water controls. However, residues aged for the same period of time when B. argentifolii nymphs were

#### TABLE 1

Treatment	Untreated leaf half: parasitoids on patch	Treated leaf half: parasitoids on patch	Untreated leaf half: parasitoids off patch	Treated leaf half: parasitoids off patch
Buprofezin	245 (178, 258)a	115 (118, 200)a	142 (48, 201)a,b	77 (34, 141)a
Pyriproxyfen	74 (5, 161)a	0 (0, 0)	195 (131, 273)a	28 (7, 65)a
Fenoxycarb	40 (7, 220)a	137 (100, 209)a	180 (21, 249)a,b	100 (36, 150)a
Pymetrozine	167 (36, 237)a	84 (16, 190)a	45 (5, 129)b	118 (38, 217)a
Kinoprene	68 (24, 151)a	2 (2, 2)b	157 (51, 243)a,b	19 (5, 138)a
Water	41 (18, 110)a	119 (89, 159)a	146 (15, 201)a,b	101 (42, 163)a

Time Spent (in Seconds) by Female *Eretmocerus eremicus* during 5-min Observation Assays Either on or off Patches of *Bemisia Argentifolii* Nymphs on Poinsettia Leaves That Were Either Treated or Untreated with Insect Growth Regulators

*Note.* Median times (untransformed) are presented with upper and lower quartiles, and values followed by the same letter within columns are not significantly different at the 0.05 level (Scheffe's means separation test).

present on leaves exhibited differences in toxicity to *E. eremicus.* This result suggests that use of whitefly nymphs treated with kinoprene and to a lesser extent pyriproxyfen and fenxoycarb for either host feeding or oviposition prolongs the toxicity of these compounds to parasitoids compared to residues of the same age on leaves when *B. argentifolii* nymphs are absent.

Immature *E. eremicus* at an early stage of development within *B. argentifolii* nymphs when IGR applications were made exhibited differences in susceptibility to compounds tested. Pymetrozine caused the lowest levels of mortality to immature parasitoids and the lowest whitefly mortality rates of tested IGRs 5 days post parasitoid oviposition. Kinoprene and pyriproxyfen caused the highest levels of immature parasitoid mortality and these two IGRs and buprofezin killed the highest proportions (>96%) of whitefly nymphs when treatments were made 5 days post parasitoid oviposition. Despite high whitefly mortality caused by IGRs, some immature parasitoids managed to survive across all treatments. This survivorship may indicate an ability of larval parasitoids to overcome adverse IGR effects on hosts because of the ability of endoparasitoids to manipulate endocrinological events in whiteflies which may utilize compounds analogous to IGRs during development. This possibility warrants further research, as it may lead to the development of IGRs that are more compatible with endoparasitoids. Immature *E. eremicus* in the late stages of development were resistant to IGR treatments and survivorship levels were similar to those seen on water-treated plants. However, unparasitized whitefly nymphs (late fourth instars and pupae) treated with IGRs 13 days post oviposition by female parasitoids exhibited significant differences in susceptibility to IGRs, with buprofezin and pyriproxyfen causing the highest levels of observed mortality.

Aphelinid parasitoids surviving buprofezin and pyriproxyfen treatments can exhibit adverse sublethal affects which are manifested as reduced longevity, fecundity, and deformed wings upon emergence (Liu and Stansly, 1997; Jones *et al.*, 1998). Furthermore, whitefly parasitoids exhibit differential susceptibility to IGRs and negative impacts are developmental stage and species dependent. For example, pyriproxyfen is safe toward immature *Encarsia pergandiella* Howard and lethal to *Encarsia formosa* Gahan ([both Hymenoptera: Aphelinidae]; Liu and Stansly, 1997). Buprofezin applied to immature *Eretmocerus tejanus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) reduces the longevity of surviving adults but a similar effect on *Eretmocerus mundus* Mercet is not observed (Jones *et al.*, 1998).

Sublethal effects of tested IGRs toward *E. eremicus* were not determined in this study. If an IGR is to be used with *E. eremicus* in a reduced parasitoid release program then reduced parasitoid longevity and fecundity could have a significant effect on whitefly control, as host feeding and parasitization rates of parasitoid progeny may be reduced following IGR applications to hosts within which parasitoids are developing. This effect could be significant, as parasitoid reproduction within greenhouses can augment weekly releases and enhance whitefly control (Hoddle *et al.*, 1997, 1998a). Consequently, further work to determine the existence of sublethal effects and to quantify adverse effects of IGRs to *E. eremicus* may be warranted.

Foraging behaviors of female *E. eremicus* were affected by dried IGR residues on poinsettia leaves when *B. argentifolii* patches were presented for oviposition and host feeding. Kinoprene and pyriproxyfen (both liquid IGRs) residues were highly repellant to parasitoids 1.5 h after drying. Following a 48-h drying period which significantly reduced leaf abandonment rates for these two compounds, E. eremicus females were not observed foraging on B. argentifolii nymphs treated with pyriproxyfen, and parasitoids spent significantly less time on kinoprene-treated leaf halves. Behavioral assays indicated that *E. eremicus* would readily abandon leaf halves to forage on non-kinoprene- and nonpyriproxyfen-treated leaf surfaces. Adverse effects on foraging behaviors of *E. eremicus* by buprofezin, fenoxycarb, and pymetrozine were not observed. The functional response of whitefly parasitoids can be altered by insecticides applied at nonlethal concentrations and foraging efficacy is affected by droplet patterns (clumped vs uniform distribution) on leaves (Perera, 1982). Integrated use of *E. eremicus* with IGRs may be enhanced by work that determines the impact of residues and IGR droplet patterns on parasitoid foraging efficacy and subsequent whitefly control.

Using the results of the work presented here, a relative ranking system (i.e., means with the same means separation letters were given the same rank within categories, ranks were then averaged, and IGRs were ranked according to average score) was used to order IGRs for compatibility with E. eremicus and for their ability to kill *B. argentifolii*. Averaging rank values for the IGRs tested on their quantified compatibility with E. eremicus and ability to kill B. argentifolii nymphs resulted in the following relative order: buprofezin >fenoxycarb > pymetrozine = pyriproxyfen > kinoprene. Buprofezin is currently unregistered for whitefly control in U.S. greenhouses. Nevertheless, further work evaluating the efficacy of this IGR for integrated use with *E. eremicus* for *B. argentifolii* on greenhousegrown poinsettias is recommended. Availability of buprofezin for concurrent use with E. eremicus would diversify whitefly control options for growers of greenhouse ornamentals, help mitigate resistance development by *B. argentifolii* to registered insecticides, and lower the cost of whitefly biological control on poinsettias.

#### ACKNOWLEDGMENTS

We thank Dr. O. Zanen, Department of Entomology, Cornell University for analyzing behavioral data. Paul Ecke Ranch, Encinitas, California provided poinsettia cuttings for experiments. Funds from the Massachusetts IPM program, Massachusetts Department of Food and Agriculture, and USDA/NRICGP Grant No. 9402481 supported this research.

#### REFERENCES

- Boukadida, R., and Michelakis, S. 1994. The use of *Encarsia formosa* in integrated programs to control the whitefly *Trialeurodes vapo-rariorum* Westw. (Hom., Aleyrodidae) on greenhouse cucumber. *J. Appl. Entomol.* **118**, 203–208.
- Dittrich, V., Uk, S., and Ernst, G. H. 1990. Chemical control and insecticide resistance of whiteflies, *In* "Whiteflies: Their Bionomics, Pest Status and Management" (D. Gerling, Ed.), pp. 263–286. Intercept Ltd., Andover, UK.
- Foltyn, S., and Gerling, D. 1985. The parasitoids of the aleyrodid *Bemisia tabaci* in Israel: Development, host preference, and discrimination of the aphelinid wasp *Eretmocerus mundus. Entomol. Exp. Appl.* **38**, 255–260.
- Gerling, D., and Sinai, P. 1994. Buprofezin effects on two parasitoids species of whitefly (Homoptera; Aleyrodidae). J. Econ. Entomol. 87, 842–846.
- Headrick, D. H., Bellows, T. S., Jr., and Perring, T. M. 1995. Behaviors of female *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aph-

elinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on sweet potato. *Environ. Entomol.* **24**, 412–422.

- Headrick, D. H., Bellows, T. S., Jr., and Perring, T. M. 1996. Behaviors of female *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on cotton, *Gossypium hirsutum*, (Malavaceae) and melon, *Cucumis melo* (Cucurbitaceae). *Biol. Control* 6, 64–75.
- Hoddle, M. S., and Van Driesche, R. G. 1996. Evaluation of *Encarsia formosa* (Hymenoptera: Aphelinidae) to control *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia (*Euphorbia pulcherrima*): A lifetable analysis. *Fla. Entomol.* **79**, 1–12.
- Hoddle, M. S., Van Driesche, R. G., and Sanderson, J. P. 1997.
  Biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia with inundative releases of *Encarsia formosa* Beltsville strain (Hymenoptera: Aphelinidae): Can parasitoid reproduction augment inundative releases? *J. Econ. Entomol.* 90, 910–924.
- Hoddle, M. S., Van Driesche, R. G., Sanderson, J. P., and Minkenberg, O. P. J. M. 1998a. Biological control of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) on poinsettia with inundative releases of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae): Do release rates affect parasitism? *Bull. Entomol. Res.* 88, 47–58.
- Hoddle, M. S., Van Driesche, R. G., Elkinton, J. S., and Sanderson, J. P. 1998b. Discovery and utilization of *Bemisia argentifolii* patches by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville strain) in greenhouses. *Entomol. Exp. Appl.* 87, 15–28.
- Hoddle, M. S., Sanderson, J. P., and Van Driesche, R. G. 1999. Biological control of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) on poinsettia with inundative releases of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae): Does varying the weekly release rate affect control? *Bull. Entomol. Res.* 89, 41–51.
- Hoddle, M. S., and Van Driesche, R. G. 1999a. Evaluation of inundative releases of *Eretmocerus eremicus* and *Encarsia formosa* Beltsville strain in commercial greenhouses for control of *Bemisia argentifolii* on poinsettia stock plants. J. Econ. Entomol. 92, 811– 824.
- Hoddle, M. S., and Van Driesche, R. G. 1999b. Evaluation of *Eret-mocerus eremicus* and *Encarsia formosa* Beltsville strain in commercial greenhouses for biological control of *Bemisia argentifolii* on colored poinsettia plants. *Fla. Entomol.* 82, 556–569.
- Horowitz, A. R., and Ishaaya, I. 1994. Managing resistance to insect growth regulators in the sweetpotato whitefly (Homoptera: Aleyrodidae). *J. Econ. Entomol.* **85**, 866–871.
- Jones, W. A., Wolfenbarger, D. A., and Kirk, A. A. 1995. Response of adult parasitoids of *Bemisia tabaci* (Hom.: Aleyrodidae) to leaf residues of selected cotton insecticides. *Entomophaga* 40, 153–162.
- Jones, W. A., Ciomperlik, M. A., and Wolfenbarger, D. A. 1998. Lethal and sublethal effects of insecticides on two parasitoids attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Biol. Control* 11, 70–76.
- Liu, T. X., and Stansly, P. A. 1997. Effects of pyriproxyfen on three species of *Encarsia* (Hymenoptera: Aphelinidae), endoparasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* **90**, 404–411.
- Perera, P. A. C. R. 1982. Some effects of insecticide deposit patterns on the parasitism of *Trialeurodes vaporariorum* by *Encarsia formosa. Ann. Appl. Biol.* **101**, 239–244.
- SAS Institute. 1989. "SAS/STAT User's Guide," Version 6, 4th ed., SAS Institute, Cary, NC.
- Van Driesche, R. G., Lyon, S. M., Hoddle, M. S., Roy, S., and Sanderson, J. P. 1999. Assessment of cost and reliability of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control in commercial poinsettia crops. *Fla. Entomol.* 82, 570–594.