

# Lethal and sublethal effects of two insect growth regulators on adult *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae)

Tong-Xian Liu<sup>a,\*</sup> and Philip A. Stansly<sup>b</sup>

<sup>a</sup> Vegetable IPM Laboratory, Department of Entomology, Texas Agricultural Experiment Station, Texas A&M University, 2415 E. Highway 83, Weslaco, TX 78596-8399, USA

<sup>b</sup> Southwest Florida Research and Education Center, University of Florida, P.O. Drawer 5127, Immokalee, FL 33934, USA

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## Abstract

Pyriproxyfen, a juvenile hormone analog, and buprofezin, a chitin synthesis inhibitor, are two insect growth regulators (IGRs) considered as selective and effective insecticides for controlling whiteflies. *Delphastus catalinae* (Horn) is a commercially produced whitefly predator considered to be a valuable biological control agent of whiteflies, especially of *Bemisia tabaci* (Gennadius) (*B. argentifolii* Bellows & Perring) in greenhouses. The compatibility of these two control strategies was tested by evaluating the lethal and sublethal effects resulting from ingestion of insect growth regulator (IGR) residues on treated *B. tabaci* eggs by adult lady beetles in the laboratory. Feeding on pyriproxyfen-treated whitefly eggs caused no significant decrease in longevity of either male or female beetles, whereas feeding on buprofezin-treated eggs reduced longevity significantly. Likewise, preoviposition period was not affected by pyriproxyfen, but was lengthened 3–6 days by buprofezin. Buprofezin reduced *D. catalinae* egg production and oviposition periods while a 28-day treatment with the low rate of pyriproxyfen actually increased these parameters. Both IGRs reduced *D. catalinae* egg fertility, especially the higher rate of pyriproxyfen and both rates of buprofezin. However, the process was largely reversed in the case of pyriproxyfen by a transfer of *D. catalinae* to water-treated whitefly eggs. Because *D. catalinae* fecundity was actually increased by 28 day exposure to the low rate of pyriproxyfen, there was no net effect on viable egg production. Thus, while both growth regulators negatively impacted *D. catalinae*, fewest side effects were observed with pyriproxyfen, and the worst of these, egg sterility, was reversible following removal of treated eggs.

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**Keywords:** Insect growth regulator; Juvenile hormone; Chitin inhibitor; *Delphastus catalinae*; Ladybeetle; Whitefly

## 1. Introduction

Pyriproxyfen and buprofezin are commonly used insect growth regulators (IGRs) against homopteran insect pests, including whiteflies (Ellsworth et al., 1997; Ishaaya and Horowitz, 1992). Pyriproxyfen is a juvenile hormone analog with relatively low mammalian toxicity that was first registered in Japan in 1991 for controlling public health pests (Miyamoto et al., 1993). The utility of pyriproxyfen in whitefly management was demonstrated based on suppression of embryogenesis and adult formation in *Bemisia tabaci* (Gennadius) (Ishaaya

and Horowitz, 1992) and *Trialeurodes vaporariorum* (Westwood) (Ishaaya et al., 1994). Buprofezin is a chitin synthesis inhibiting growth regulator that has been effectively used to control *B. tabaci* (= *B. argentifolii* Bellow & Perring) on cotton and other crops (Ishaaya et al., 1988; Horowitz and Ishaaya, 1994; Ishaaya and Horowitz, 1992).

Insect growth regulators are generally considered compatible with natural enemy conservation. Buprofezin was found relatively innocuous to some hymenopteran parasitoids, including *Eretmocerus* spp. and *Encarsia* spp., parasitoids of whiteflies, and an aphid parasitoid, *Lysiphlebus testaceipes* (Cresson) (Castaner and Garrido, 1995; Gerling and Sinai, 1994). It was also deemed harmless to larvae and eggs of *Chrysoperla*

\* Corresponding author. Fax: 1-956-968-0641.

E-mail address: [tx-liu@tamu.edu](mailto:tx-liu@tamu.edu) (T.-X. Liu).

*externa* Hagen and *C. rufilabris* (Burmeister) (Liu and Chen, 2000; Velloso et al., 1997), and to have no side effects on several species of mirids (Castaner and Garrido, 1995). Likewise, McMullen (1990) found that pyriproxyfen was safe to predators of pear psylla (*Psylla pyricola* Forester), such as *Anthocoris antevolens* White, *A. nemoralis* (F.), *Deraeocoris verbasci* Uhler, *Campylomma verbasci* (Meyer-Dür), and *Chrysopa* spp. Liu and Stansly (1997) found few detrimental effects of pyriproxyfen on *Encarsia* spp. with the exception of *E. formosa* (Gahan). No effects were seen on *C. rufilabris* (Chen and Liu, 2000), or several species of scale parasitoids (Peleg, 1988).

In contrast, reported effects on coccinellids from feeding on buprofezin or pyriproxyfen-treated prey are far from innocuous. In general, chitin inhibitors cause sterilization of insect adults as well as mortality at every molt of larvae or nymphs, whereas the juvenile hormone analogues also cause sterilization of adults, but mortality only at pupation or adult emergence (Hattingh and Tate, 1995; Magagula and Samways, 2000; Mendel et al., 1994; Smith, 1995). The loudest alarm was sounded by Hattingh and Tate (1995) when they reported secondary outbreaks of cottony cushion scale, *Icerya purchasi* (Maskell), due to sterilization of the coccinellid *Rodolia cardinalis* (Mulsant) following use of pyriproxyfen for the control of red scale, *Aonidiella aurantii* (Maskell), on citrus in southern Africa. Side effects from IGRs on *R. cardinalis* were previously reported by Loia and Viggiani (1992) and Mendel et al. (1994). Impacts of IGRs have also been reported on the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Smith et al., 1999), and on key predators of armored scales such as the coccinellids *Chilocorus nigrita* (F.) (Hattingh and Tate, 1995; Magagula and Samways, 2000), *C. bipustulatus* (L.) (Erkilic and Uygun, 1997; Loia and Viggiani, 1992; Mendel et al., 1994; Peleg, 1983), *C. circumdatus* Gyllenhal (Smith, 1995; Smith et al., 1999), and the nitidulid predator *Cybocephalus fodori* Endrödy-Younga (Erkilic and Uygun, 1997).

*Delphastus catalinae* (Horn), often misidentified as *D. pusillus* (LeConte) (Gordon, 1994; Hoelmer and Pickett, 2003), is reported to have the ability to control whiteflies, including *B. tabaci* biotype “B” and *Trialetrodes vaporariorum* (Westwood) on ornamental and vegetable crops under greenhouse conditions (Heinz et al., 1994; Hoelmer et al., 1993) and is commercially available in the US, Canada, and elsewhere (Hunter, 1998). However, there is no published information on possible effects of IGRs on this or other coccinellid predators of whiteflies. Integration of biological and chemical control requires knowledge of the effects of insecticides on natural enemies. Therefore, there is a need for information of the effects of these insect growth regulators on *D. catalinae*. Our objective was to evaluate the gravity and persistence of effects on adult survivorship and fecundity

of *D. catalinae* resulting from exposure to the two most commonly used insect growth regulators for whitefly control.

## 2. Materials and methods

### 2.1. Insect growth regulators

Pyriproxyfen (Knack 0.86 EC [emulsifiable concentrate], Valent USA, Walnut Creek, CA, USA) and buprofezin (Applaud 70 WP [wetttable powder], Bayer, Kansas City, MO, USA) were used at 0.1 and 0.2 g (AI)/liter, respectively. The concentrations for both IGRs were based on recommended field rates. Purified water was used as a control in all treatments.

### 2.2. Insects and host plants

*Delphastus catalinae* were originally supplied by Arizona Biological Control Inc. (Tucson, AZ) in 1998, and were maintained in a greenhouse at the Vegetable IPM Laboratory, Texas A&M University Agricultural Research and Extension Center at Weslaco, Texas, USA. Collard (*Brassica oleracea* L. var. *acephala*, ‘Georgia LS’) was used as the host plant for the prey, *B. tabaci*. The collard plants were grown in 15-cm plastic pots filled with Metro-Mix7 300 growing medium (Grace Sierra, Horticultural Products Company, Milpitas, CA), to which sufficient slow release fertilizer (N-P-K: 12-8-6) was added as needed to maintain normal growth. Uninfected collard plants were exposed to whiteflies for 24 h to obtain eggs for treatments. Voucher specimens of *D. catalinae* were deposited at the Insect Collection of the Texas Agricultural Experiment Station, Texas A&M University, Weslaco, TX, and the Insect Collection, Southwest Florida Research and Education Center, University of Florida, Immokalee, FL, USA.

### 2.3. Lethal and sublethal effects on adults

Egg bearing collard leaves were sprayed with appropriate dilutions of the IGRs using a hand-held sprayer until runoff, air dried for 1–2 h and stored in zip-lock plastic bags for no more than 1 week in a refrigerator at 5–6 °C. Beetles 24-h old were collected from the laboratory colony and pairs (male/female) fed individually in a petri dish with the IGR-treated whitefly eggs on 7–8 cm leaf disks containing  $\geq 30$  eggs/cm<sup>2</sup>. Leaf disks were replaced and beetles were monitored daily until all had died. If the male of the pair died within 10 days, a new male was added. The observation was terminated and data were not used if the female of the pair died within 10 days. Ten pairs of *D. catalinae* adults (1 female and 1 male) were initially used in each IGR treatment. Data from 10 pairs of *D. catalinae* fed water-treated

whitefly eggs were used as controls for both IGRs. To determine if sterilization of *D. catalinae* females caused by the IGRs was reversible, 7–8 additional pairs from each treatment were fed with water-treated whitefly eggs starting from day 29 until all females died.

Numbers of eggs deposited by each female beetle were counted and examined as soon as the leaf disk was removed from the petri dish. Disks bearing *D. catalinae* eggs were then individually incubated in petri dishes in an insectary at  $25 \pm 2^\circ\text{C}$ , 50–60% RH, and room lighting. They were examined daily until either all beetle eggs hatched or died. The following parameters were obtained from the adult assays: preoviposition period, longevity, fecundity, oviposition period (number of days from the first to last egg), daily oviposition per female, days with no oviposition, abnormal eggs lacking a chorion, and viable eggs from which larvae eventually hatched.

#### 2.4. Data analysis

Percentages of *D. catalinae* viable eggs and no-chorion eggs were arc sine square root [arc sine  $\sqrt{(\text{percent mortality})}$ ] transformed before analysis to stabilize error variance (Gomez and Gomez, 1984), although untransformed data are given in tables and figures. Treatment

effects on adult longevity, fecundity, oviposition, and other data were analyzed using two-way analysis of variance (ANOVA), and means were separated using the least significant difference (LSD) test following a significant *F*-test (SAS Institute, 2003).

### 3. Results

#### 3.1. Adult longevity

Male *D. catalinae* lived an average of 53.9 days longer than females when feeding on water-treated whitefly eggs ( $F = 15.05$ ;  $df = 1, 38$ ;  $P = 0.0004$ ) (Fig. 1). Feeding on buprofezin-treated eggs significantly decreased fecundity of both sexes compared with those treated with pyriproxyfen and water control (female:  $F = 4.32$ ;  $df = 2, 19$ ;  $P = 0.0478$ ; male:  $F = 10.18$ ;  $df = 2, 19$ ;  $P = 0.0010$ ), with no significant difference between the two concentrations applied (Fig. 1). However, the effect disappeared when beetles were transferred to water-treated whitefly eggs after 29 days ( $F = 0.20$  and  $3.19$ ;  $df = 2, 19$ ;  $P = 0.0693$  and  $0.8112$  for female and male, respectively). Similar trends were observed for beetles kept on a diet of pyriproxyfen-treated whitefly eggs, although differences were not significant. The longevity

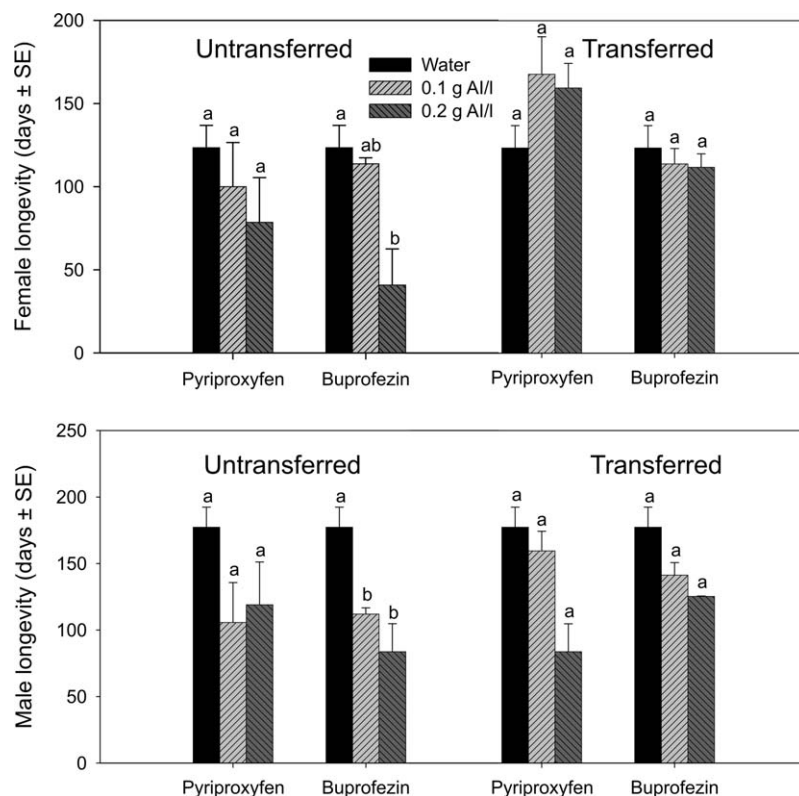


Fig. 1. Longevity of *Delphastus catalinae* adults feeding on *B. tabaci* eggs on collard leaves treated with pyriproxyfen, buprofezin, or water. Untransferred: fed with treated whitefly eggs until death. Transferred: the adults were fed with pyriproxyfen- or buprofezin-treated whitefly for 28 days, and were subsequently fed with water-treated whitefly eggs.

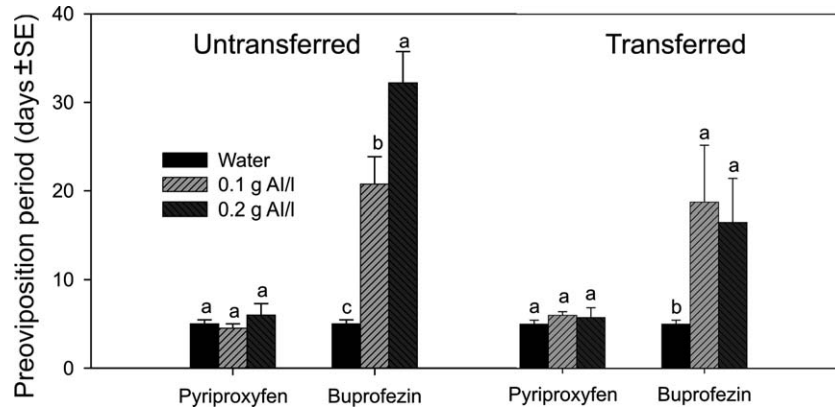


Fig. 2. Preoviposition periods of *Delphastus catalinae* females after feeding on pyriproxyfen-, buprofezin-, or water-treated *Bemisia tabaci* eggs on collard leaf disks. Untransferred: fed with treated whitefly eggs until death. Transferred: the adults were fed with pyriproxyfen- or buprofezin-treated whitefly for 28 days, and were subsequently fed with water-treated whitefly eggs.

of females actually increased with respect to the untreated control when transferred to untreated whitefly eggs after 29 days ( $F = 4.08$ ;  $df = 1, 19$ ;  $P = 0.0463$ ). No such effect was observed with males ( $F = 1.91$ ;  $df = 1, 19$ ;  $P = 0.1757$ ).

### 3.2. Preoviposition and oviposition periods

A steady diet of buprofezin-treated whitefly eggs significantly increased preoviposition period four times at the low rate and eight times at the high rate (Fig. 2,  $F = 6.69$  and  $55.54$ ;  $df = 2, 16$ ;  $P = 0.0084$  and  $<0.0001$ , respectively). In contrast, no effect on preoviposition period of *D. catalinae* was observed by pyriproxyfen at either rate ( $F = 0.76$  and  $0.89$ ;  $df = 2, 19$ ;  $P = 0.4262$  and  $0.4842$ , respectively).

Buprofezin significantly decreased oviposition period and oviposition days for both transferred and untransferred *D. catalinae* females (Table 1). Females fed continuously on whitefly eggs treated with the higher rate of buprofezin oviposited only twice during a 37-day period; even the lower rate reduced oviposition days an order of magnitude. Both oviposition period and oviposition days of *D. catalinae* females were decreased by a steady

diet of pyriproxyfen-treated whitefly eggs although differences were not significant in the case of oviposition period (Table 1). Here again, the trend was reversed, although not significantly, for *D. catalinae* adult females that fed pyriproxyfen-treated eggs and were transferred to water-treated eggs after 29 days (Table 1). *D. catalinae* oviposition period was significantly longer for beetles transferred to water-treated whitefly eggs compared with untransferred beetles ( $F = 2.40$ ;  $df = 2, 15$ ;  $P = 0.1173$ ).

### 3.3. Fecundity and fertility

Buprofezin greatly reduced *D. catalinae* fecundity, especially for beetles maintained indefinitely on treated whitefly eggs (Figs. 3 and 4). Even the low rate reduced total oviposition to less than 10 eggs (Table 2). Transferring *D. catalinae* to untreated eggs at 29 days partially restored the ability to oviposit in the beetles receiving the low rate. In contrast, pyriproxyfen caused no significant reduction in oviposition (Figs. 3 and 4, Table 2). Mean fecundity increased significantly if beetles were transferred to water-treated whitefly eggs after 29 days ( $F = 3.42$ – $7.91$ ;  $df = 1, 8$ ;  $P = 0.0227$ – $0.0102$ ).

Table 1  
Oviposition period and oviposition days of *Delphastus catalinae* females fed pyriproxyfen-, buprofezin-, or water-treated *Bemisia tabaci* eggs

Rate (g AI/l)	Fed pyriproxyfen-treated eggs				Fed buprofezin-treated eggs			
	Untransferred		Transferred <sup>a</sup>		Untransferred		Transferred	
	Oviposition period <sup>b</sup>	Oviposition days	Oviposition period <sup>b</sup>	Oviposition days	Oviposition period <sup>b</sup>	Oviposition days	Oviposition period <sup>b</sup>	Oviposition days
0.1	76.0 ± 18.2	46.3 ± 14.3b	122.3 ± 14.3	101.0 ± 12.1	34.0 ± 12.9b	7.8 ± 3.3b	58.8 ± 9.8b	24.3 ± 15.3b
0.2	51.8 ± 15.6	42.8 ± 9.4b	101.0 ± 7.2	83.0 ± 6.4	37.0 ± 11.1b	2.0 ± 3.2b	57.3 ± 9.8b	17.3 ± 3.8b
Water	90.8 ± 7.6	77.7 ± 6.8a	90.8 ± 7.6	77.7 ± 6.8	90.8 ± 7.6a	77.7 ± 6.8a	90.8 ± 7.6a	77.7 ± 6.8a
<i>F</i>	2.40	4.43	2.55	0.07	11.43	57.86	4.77	17.53
<i>P</i>	0.1173	0.0263	0.1110	0.9322	0.0006	<0.0001	0.0249	<0.0001

<sup>a</sup> The adults were fed with IGR-treated whitefly eggs for 28 days and then were fed with water-treated whitefly eggs.

<sup>b</sup> Means in the same column followed by the same letters are not significantly different at  $P = 0.05$  (LSD, SAS Institute, 2003).

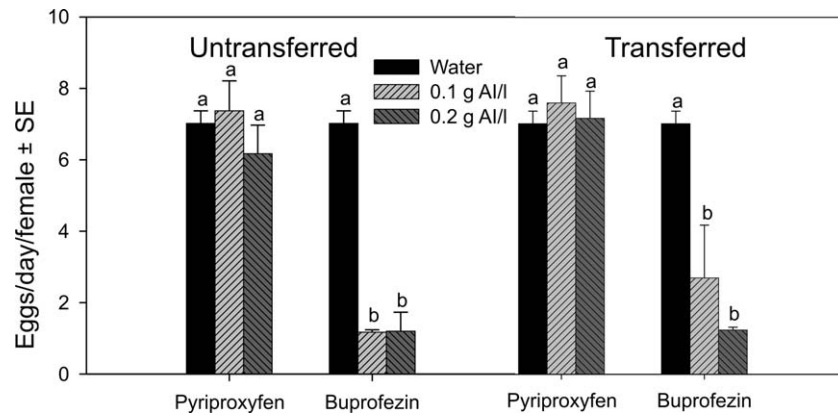


Fig. 3. Overall mean eggs deposited per day by *Delphastus catalinae* females after feeding on pyriproxyfen-, buprofezin-, or water-treated *Bemisia tabaci* eggs on collard leaf disks. Untransferred: fed with treated whitefly eggs until death. Transferred: the adults were fed with pyriproxyfen- or buprofezin-treated whitefly for 28 days, and were subsequently fed with water-treated whitefly eggs.

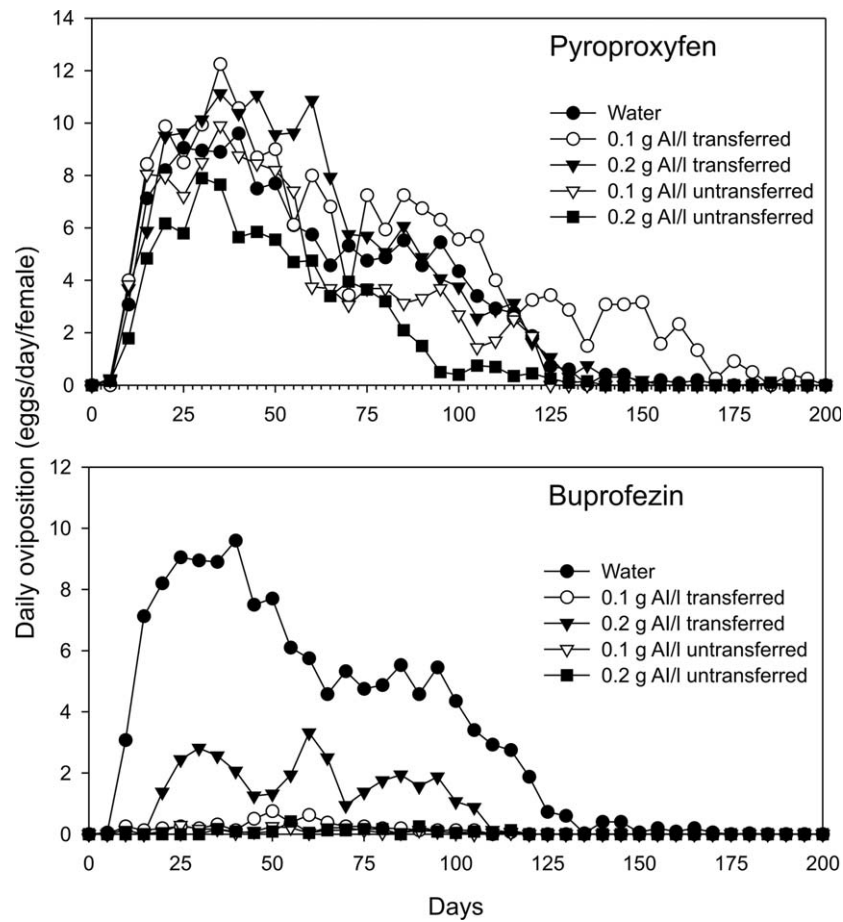


Fig. 4. Daily oviposition of *Delphastus catalinae* that were fed with pyriproxyfen-, buprofezin-, or water-treated whitefly eggs. Untransferred: the adults were fed with treated whitefly eggs until they died. Transferred: the adults were fed with pyriproxyfen- or buprofezin-treated whitefly for 28 days, and were subsequently fed with water-treated whitefly eggs.

Both IGRs caused drastic reductions in *D. catalinae* egg viability (Table 2). Virtually no *D. catalinae* eggs hatched at the high rate of either material. Sterile beetle eggs were often abnormal in appearance, being soft and

semi-fluid, without a hardened or complete chorion. Buprofezin also effectively sterilized *D. catalinae* males by causing the aedeagus to protrude from the tip of abdomen rendering it nonfunctional. Pyriproxyfen-treated

Table 2

Numbers of eggs deposited by the *Delphastus catalinae* females that were fed with pyriproxyfen-, buprofezin- or water-treated *Bemisia tabaci* eggs, and percentage of viable eggs (or larval hatch rate)

Rate (g AI/l)	Fed pyriproxyfen-treated eggs				Fed buprofezin-treated eggs			
	Untransferred		Transferred <sup>a</sup>		Untransferred		Transferred	
	Total eggs deposited <sup>b</sup>	% viable eggs	Total eggs deposited	% viable eggs	Total eggs deposited	% viable eggs	Total eggs deposited	% viable eggs
0.1	376.3 ± 133.0	35.0 ± 8.5b	722.8 ± 109.6	86.0 ± 1.4b	9.2 ± 3.9b	5.8 ± 3.6b	133.0 ± 21.7b	16.8 ± 14.7b
0.2	285.3 ± 91.7	0.3 ± 0.2c	631.8 ± 57.0	72.0 ± 2.1c	8.0 ± 2.1b	0.0 ± 0.0b	22.0 ± 5.5b	0.0 ± 0.0b
Water	541.2 ± 50.7	95.9 ± 0.9a	541.2 ± 50.7	95.9 ± 0.9a	541.2 ± 50.7	95.9 ± 0.9a	541.2 ± 50.7a	95.9 ± 0.9a
F	2.46	159.78	1.78	104.96	63.40	942.71	17.96	96.54
P	0.1125	<0.0001	0.2024	<0.0001	<0.0001	<0.0001	0.0001	<0.0001

<sup>a</sup> The adults were fed with IGR-treated whitefly eggs for 28 days and then were fed with water-treated whitefly eggs.

<sup>b</sup> Means in the same column followed by the same letters are not significantly different at  $P = 0.05$  (LSD, SAS Institute, 2003).

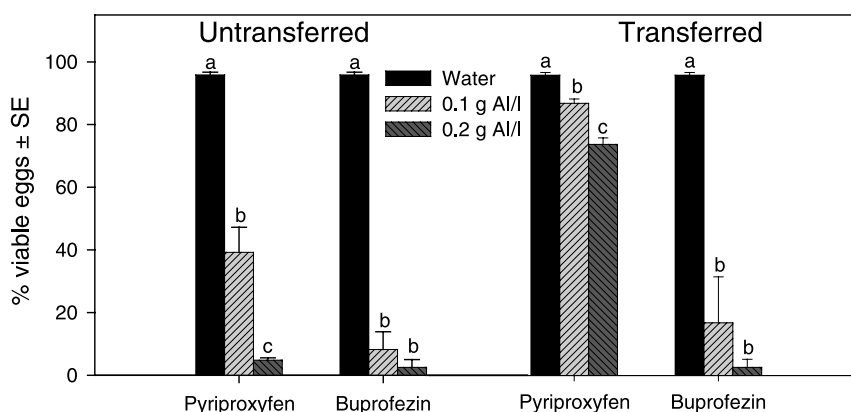


Fig. 5. Overall egg viability deposited by *Delphastus catalinae* that were fed with pyriproxyfen-, buprofezin-, or water-treated whitefly eggs. Untransferred: the adults were fed with treated whitefly eggs until death. Transferred: the adults were fed with pyriproxyfen- or buprofezin-treated whitefly for 28 days, and were subsequently fed with water-treated whitefly eggs.

*D. catalinae* females recuperated when transferred to untreated whitefly eggs at 29 days whereas those treated with buprofezin did not. In fact, the expected number of viable eggs from a transferred female *D. catalinae* (fecundity × fertility) was 622 compared with 519 for an untreated female Fig. 5.

#### 4. Discussion

Our results show little compatibility between *D. catalinae* and the use of buprofezin. Feeding on whitefly eggs treated with this chitin inhibitor decreased longevity, increased preoviposition period and had a devastating effect on fecundity and fertility of *D. catalinae*. This was not reversed when the same adults were then fed untreated whitefly eggs. This is in contrast to rapid reversibility of sterilization in *C. bipustulatus* by the chitin inhibitor diflubenzuron following transfer to untreated armored scale (Peleg, 1983) and partial recovery of egg viability within 5–10 days following removal of buprofezin-sprayed citrus leaves (Hattingh and Tate, 1995). These latter authors also reported shorter per-

sistence of buprofezin residues compared to pyriproxyfen. Nevertheless, given the persistent sterilization we observed coupled with likely effects on larvae and/or pupae extrapolated from reports on other ladybeetle species (Magagula and Samways, 2000; Mendel et al., 1994; Smith, 1995), buprofezin should be used with caution where whitefly control depends on *D. catalinae*.

The effects on pyriproxyfen on adult *D. catalinae* were largely reversible and therefore might be easier to manage. *D. catalinae* egg viability returned to almost normal after transfer to untreated whitefly eggs, and fecundity, if any, increased due to extended longevity. This is the first report of restored egg viability following transfer of a coccinellid from pyriproxyfen-treated prey to untreated prey. However sterilization was reversed in *C. nigrita* 20 days after removal of treated citrus leaves, and in *C. bipustulatus* following transfer to untreated scale insects after 3 weeks feeding on scale insects treated with the juvenile hormone analogues methoprene and fenoxycarb (Peleg, 1983).

Exposure of *D. catalinae* to spray residues on collards under laboratory conditions is likely to be considerable shorter than exposure of ladybeetles on perennial crops

such as citrus where field-weathered residues may remain active for weeks or even months (Hattingh and Tate, 1995; Smith et al., 1999). Such residues might even persist longer in treated scale insects that serve as prey for these beetles. In contrast, *D. catalinae* feeds primarily on whitefly eggs (Liu and Stansly, 1999; Liu, unpublished data). Target whiteflies typically infest fast-growing herbaceous crops and prefer young foliage for oviposition. The rapid appearance of new foliage and untreated prey would facilitate the use of either IGR, but especially pyriproxyfen due to the reversibility of sterilization.

Hoddle et al. (2001) ranked the compatibility of IGRs to the whitefly parasitoid *Eretmocerus eremicus* Rose and Zolnerowich as buprofezin > fenoxycarb > pyriproxyfen > kinoprene, and Van Driesche et al. (2001) showed how two mid-season applications of buprofezin could be integrated with releases of *E. eremicus* to control whiteflies on greenhouse poinsettias. Ranking pyriproxyfen as more compatible than buprofezin to *D. catalinae*, how could these IGRs be used if *D. catalinae* were added to the system? Parasitoids such as *Eretmocerus mundus* Mercet are generally more effective at low host densities than ladybeetles, but must be established early in a relatively pesticide-free environment (Stansly et al., 2004). Parasitoid releases could be followed by releases of ladybeetles later in the crop if whitefly densities were to increase. These could be followed in turn with IGRs if whitefly populations continued to increase, the choice depending on which biological agent is exerting most control at the time. Buprofezin would have the least effect on parasitoids while pyriproxyfen, although temporarily sterilizing *D. catalinae* would not reduce longevity or oviposition. Thus, the availability of selective pesticides such as the IGRs increases both the opportunities for integrating chemical and biological control, as well as the complexity of the resulting management systems.

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