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

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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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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* Bellows & 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 homopteran 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*...
Chilocorus nigrita, such as the coccinellids (Castaner and Garrido, 1995). Likewise, McMullen (1990) found that pyriproxyfen was safe to predators of pear psylla (Psylla pyricola Forrester), such as Anthocoris antevolens White, A. nemoralis (F.), Deraeocoris verbesi Uhler, Campylomma verbasci (Meyer-Dür), and Chrysopea 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 parasites (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 coccinell Rodolia cardinalis (Mulsant) following use of pyriproxyfen for the control of red scale, Aonidiella aurantii (Mulsant) (Smith, 1995; Smith et al., 1999), or several species of scale parasioids (Peleg, 1988).

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 [wettable 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 ºC. 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 30 eggs/cm². 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 ± 2°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 \((F = 15.05; \text{df} = 1, 38; P = 0.0004)\) (Fig. 1). Feeding on buprofezin-treated eggs significantly decreased fecundity of both sexes compared with those treated with pyriproxyfen and water control (female: \(F = 4.32; \text{df} = 2, 19; P = 0.0478\); male: \(F = 10.18; \text{df} = 2, 19; P = 0.0010\)), 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 \((F = 0.20 \text{ and } 3.19; \text{df} = 2, 19; P = 0.0693 \text{ and } 0.8112 \text{ for female and male, respectively})\). 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.](image-url)
of females actually increased with respect to the un-
treated control when transferred to untreated whitefly
eggs after 29 days \((F = 4.08; \text{df} = 1, 19; P = 0.0463)\). No
such effect was observed with males \((F = 1.91; \text{df} = 1, 19; P = 0.1757)\).

### 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; \(\text{df} = 2, 16; P = 0.0084\) 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; \(\text{df} = 2, 19; P = 0.4262\)
and 0.4842, respectively).

Buprofezin significantly decreased oviposition period
and oviposition days for both transferred and untrans-
ferred \(D.\) catalinae females (Table 1). Females fed con-
tinuously 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 dif-
fferences 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). \(D.\) catali-
nae oviposition period was significantly longer for bee-
tles transferred to water-treated whitefly eggs compared
with untransferred beetles \((F = 2.40; \text{df} = 2, 15;
\ P = 0.1173)\).

### 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). Trans-
ferring \(D.\) 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 sig-
nificant 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
\((F = 3.42–7.91; \text{df} = 1, 8; P = 0.0227–0.0102\).

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**Table 1**

<table>
<thead>
<tr>
<th>Rate (g AI/l)</th>
<th>Fed pyriproxyfen-treated eggs</th>
<th>Fed buprofezin-treated eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untransferred</td>
<td>Transferred</td>
</tr>
<tr>
<td></td>
<td>Oviposition period</td>
<td>Oviposition days</td>
</tr>
<tr>
<td>0.1</td>
<td>76.0 ± 18.2</td>
<td>46.3 ± 14.3b</td>
</tr>
<tr>
<td>0.2</td>
<td>51.8 ± 15.6</td>
<td>42.8 ± 9.4b</td>
</tr>
<tr>
<td>Water</td>
<td>90.8 ± 7.6</td>
<td>77.7 ± 6.8a</td>
</tr>
<tr>
<td>(F)</td>
<td>2.40</td>
<td>4.43</td>
</tr>
<tr>
<td>(P)</td>
<td>0.1173</td>
<td>0.0265</td>
</tr>
</tbody>
</table>

*The adults were fed with IGR-treated whitefly eggs for 28 days and then were fed with water-treated whitefly eggs.

*Means in the same column followed by the same letters are not significantly different at \(P = 0.05\) (LSD, SAS Institute, 2003).
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...
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/C2 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 persistence 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 transferal 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.

Hoddlie et al. (2001) ranked the compatibility of IGRs to the whitefly parasitoid Eremocerus eremicus Rose and Zolnerovich 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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