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IMPACT OF METHOPRENE AND PYRIPROXYFEN ON *PSEUDACTEON TRICUSPIS* (DIPTERA: PHORIDAE), A PARASITOID OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Endoparasitoid phorid flies, *Pseudacteon* spp. (Diptera: Phoridae) are important biological control agents of imported fire ants *Solenopsis invicta* Buren, and *S. richteri* Forel (Hymenoptera: Formicidae). The impact on phorid flies by insecticides, particularly insect growth regulators, used in controlling imported fire ants has yet to be explored. Red imported fire ants parasitized by *Pseudacteon tricuspis* Borgmeier were exposed to methoprene and pyriproxyfen, the active ingredients used in some fire ant baits. These chemicals are insect growth regulators (IGRs), which affect the reproductive capabilities of the colony, but have no acute toxicity to fire ant workers. An experiment tested the effects of the 2 IGRs on the phorid fly larva at 2 time intervals (d 6 and 10 post-parasitism), when the larva was present in the thorax and head of the adult ant host, respectively. The mean proportion of emerged *P. tricuspis* from methoprene and pyriproxyfen treatments was significantly reduced relative to the control. Timing of exposure to the IGRs (ds post-parasitism) did not have a significant impact on the emergence of the phorid fly.

Key Words: biological control, insect growth regulators

RESUMEN

Las moscas endoparasitoides de la familia Phoridae, *Pseudacteon* spp. (Diptera: Phoridae) son agentes de control biológico importantes de la hormiga de fuego roja importada, *Solenopsis invicta* Buren, y *S. richteri* Forel (Hymenoptera: Formicidae). El impacto de los insecticidas, particularmente los reguladores del crecimiento de los insectos, sobre las moscas fóridas, que se usan para controlar la hormiga de fuego roja importada, todavía no han sido explorados. La hormiga de fuego roja importada parasitizadas por *Pseudacteon tricuspis* Borgmeier fueron expuestas a metoprena y piriproxifen, los ingredientes activos usados en algunos de los cebos para la hormiga de fuego. Estos químicos son reguladores del crecimiento de los insectos (RCI), que afectan la capacidad reproductiva de la colonia, pero no tienen toxicidad aguda a los trabajadores de la hormiga de fuego. Un experimento probó los efectos de los 2 RCI sobre larvas de la mosca fórida en 2 intervalos de tiempo (6 y 10 días pos-parasitismo), cuando las larvas fueron presentes en el tórax y cabeza de los adultos de las hormigas hospederas, respectivamente. El promedio de la proporción de *P. tricuspis* que emergieron en los tratamientos con metoprena y piriproxifen fue significativamente reducido en relación con el control. El tiempo cuando las moscas fóridas fueron expuestas a los RCI (días pos-parasitismo) no tuvo un impacto significativo sobre la emergencia de las moscas fóridas.

Since their introduction from South America in the 1930s, red imported fire ants, *Solenopsis invicta* Buren have spread to 11 southeastern states, Puerto Rico (Callcott & Collins 1996), and parts of New Mexico and California. Imported fire ants are not only a direct pest to humans; they also affect wildlife, disrupt electrical systems, cause losses in agriculture, and have an economic impact through the cost of control and quarantine. The economic impact of imported fire ant control in infested states exceeds \$5 billion annually (Lard et al. 2006). Environmentally friendly formulations of baits were explored in the 1970s and 1980s after decades of unsuccessful attempts at eradication, which were eventually halted due to use of chemicals that persisted in the environ-

ment and had non-target effects (Williams et al. 2001). Baits are advantageous because they allow for smaller amounts of active ingredient per unit area, have lower production costs, and are more selective. There are 2 main types of baits: toxins, which kill all castes within the colony, and insect growth regulators (IGRs), which target the reproductive (queen) capabilities of the colony, with no acute effect on the sterile female worker ants (Banks 1986). Methoprene and pyriproxyfen are among the most commonly used IGRs for imported fire ant control (Oi & Drees 2009).

Because imported fire ants arrived in the U.S. without their natural enemies (Jouvenaz et al. 1977), efforts were made to find and import such biological controls. In 1995, phorid flies of the

Pseudacteon genus were imported as classical biological control agents against the red imported fire ant. Of the 18 species known to attack ants of the *Solenopsis saevissima* complex in South America (Porter & Pesquero 2001; Folgarait et al. 2005), 4 species have since been released in the U.S.: *P. litoralis* Borgmeier, *P. curvatus* Borgmeier, *P. tricuspsis* Borgmeier, and *P. obtusus* Borgmeier. Their benefit is twofold in that they kill (decapitate) worker ants (Porter et al. 1995a; Consoli et al. 2001), and, more importantly, reduce colony health (Folgarait & Gilbert 1999) by altering foraging behavior (Feener & Brown 1992; Orr et al. 1995; Porter et al. 1995b; Mehdiabadi & Gilbert 2002). Each *Pseudacteon* species fills a specific niche, with differences in time of activity and host size preference (Campiolo et al. 1994; Fowler et al. 1995; Pesquero et al. 1996; Morrison et al. 1997; Morrison & Gilbert 1998; Folgarait et al. 2003).

Development of the phorid fly starts when an adult female oviposits one egg into the thorax of the worker ant. The egg hatches, and the larva migrates from the thorax to the head, developing through three instars. The last instar “decapitates” the head of the ant by releasing an enzyme, which causes degradation of the connective tissue, prior to pupation. The ant’s head then serves as a puparium (Porter et al. 1995a) from which an adult phorid fly emerges 4 to 6 weeks later (Consoli et al. 2001).

Interactions of insect growth regulators and parasitoids have been documented in many species, although no reports were found on the impact of IGRs on *Pseudacteon* spp. The objective of this study was to determine the potential impact of methoprene and pyriproxyfen on developing *P. tricuspsis* larvae.

MATERIALS AND METHODS

Ants parasitized by *P. tricuspsis* were obtained from the Florida Department of Agriculture and Consumer Services, Biological Control Rearing Facility in Gainesville, FL. At the Florida lab, ants were sieved from a stock of field collected ants that ranged in size from 1.6 to 5 mm. A size 20 sieve separated ants to the preferred host size used for parasitization by *P. tricuspsis*. Once separated, ants were exposed to attack by *P. tricuspsis* for 4 d. The following day, a shipment of at least 6 g of live ants (600 ants/g) was shipped to the University of Arkansas lab (Fayetteville, AR) via an overnight carrier. To minimize ant mortality, personnel at the rearing facility advised reducing stress to the ants by limiting handling and disruptions, use of a water tube, and adding a small castone block (dental grade castone mixed in a ratio of 100 g to 33 mL water, cast in a flexible ice cube tray with an indentation for supplying water) per container.

Upon receiving the shipment, ants were provided ample distilled water to prevent desiccation and maintained at $27 \pm 1.8^\circ\text{C}$, a humidity $\geq 60\%$, and a photoperiod of 8:16 (L:D) (8:00 AM to 4:00 PM CST). Following a 24-h acclimation, ants were transferred into 6 holding containers (Sterilite® plastic shoebox #1851, 32 by 17.5 by 11 cm) with a 0.3-cm wooden dowel. Rectangular holes were cut in the container lids and chiffon adhered to the lid opening to provide ventilation from above. The top 11 cm of the container were lined with Fluon® to prevent escape of worker ants. Each container included a castone block, a water wicked tube (2- mL shell vial filled with distilled water and wicked with a piece of a cotton ball), a nesting tube (16- by 150-mm test tube with the bottom quarter filled with distilled water followed by a cotton ball and 5 mm of castone), and a sugar source (white granulated sugar saturated (1400 g sugar to 2 L water) 1 ply Kimwipes™ (11 by 21 cm) rolled into balls and allowed to dry in an incubator and re-hydrated when placed with the ants). The water tube and sugar source were replaced every other d, the castone block was saturated daily, and the nesting tube was replaced upon evidence of mold, feeding by ants or significant water loss.

Preliminary Work

Because temperature, humidity, and photoperiod are important factors in the development rate of *P. tricuspsis* (Consoli et al. 2001; Morrison et al. 1997; Porter et al. 1997), parasitized ants were dissected to determine the stage and location within the host. From 6 Jun through 23 Jun 2009 and beginning 2 d post-parasitization, a minimum of 10 ants were dissected daily. These data and review of the literature (Consoli et al. 2001) were used to determine the appropriate day post-parasitization to expose the parasitized ants to the insect growth regulators when developing *P. tricuspsis* were in the head or thorax. Based on this information, d 6 and 10 post-parasitization was chosen to ensure ant exposure to IGRs when *P. tricuspsis* larvae were found in the thorax and head, respectively. Initially, parasitized ants were exposed to the IGR in commercial baits Esteem® (0.5% A.I. pyriproxyfen) and Extinguish® (0.5% A.I. methoprene). Rates were determined by assuming 1 g of bait provided to 400 ants would be approximately 4% of the recommended individual mound treatment rate (20 g) to a colony with 10,000 ants. Bait granules were placed in vial lids within the treatment containers to allow the ants to feed for 12 h. However, limited feeding was observed, with only 10% of the ants feeding on the baits. Subsequent modifications included removing any water source during feeding, and withholding water and food for 12 h prior to feeding.

Because limited feeding was observed and equal exposure of all ants was uncertain, another method of ant exposure was developed with treated filter papers (Dean & Meola 1997). Technical grade pyriproxyfen and methoprene (Chem Service, Inc., West Chester, PA) were mixed into solution with 99.5% acetone. Based on their percent purity, calculations were made to produce a rate (4,940 $\mu\text{g}/\text{mL}$) comparable to the amount active ingredient in 1 g of formulated bait (5,000 μg). Tests were conducted with the 2 chemicals at d 6 and d 10 post-parasitization.

IGR Exposure

Four replications were evaluated from Jun 2009 to Aug 2009. Parasitized ants, obtained from the Florida lab were divided into 6 treatment groups, 400 ants per container: ants that were exposed to pyriproxyfen on d 6 (P6) and 10 (P10), ants that were exposed to methoprene on d 6 (M6) and 10 (M10), and ants that were exposed to acetone control on d 6 (C6) and 10 (C10).

On the day of treatment, 1 mL of 4,940 $\mu\text{g}/\text{mL}$ solution was applied to a 9-cm diameter filter paper. Controls were prepared with acetone only. Filter papers were allowed to dry in a fume hood for ≥ 2 h before being transferred to a plastic Petri dish. Fluon[®] was applied to the inner rim of the bottom half of a plastic Petri dish (100 by 15 mm). Ants from the treatment group were placed into the bottom half; the lid with the treated filter paper placed on top and the whole component inverted. The top of the Petri dish was tapped to dislodge any ants from the upper part. Two 1 kg weights were placed on the Petri dish container to create a tight seal for the entire 6-h exposure time.

Upon reintroduction to the holding container, ants were provided a new sugar source and water tube. Immediately following the 6-h exposure, dead ants were removed. Dead ants were removed daily from all treatments. Dead ants were counted and ant heads separated and placed into individual portion cups (Comet[™] P10BAGREV1 clear 1 fl oz). Two holes (0.7 mm diameter) were punched in the portion cup lid to provide air exchange. Chiffon screen was placed between the portion cup and lid to prevent escape of any emerged flies. Ant heads were measured across the lower margin of the eyes (Morrison et al. 1997) to the nearest thousandth millimeter with an ocular micrometer affixed to a dissecting microscope (Nikon SMZ1000). Portion cups containing decapitated ant heads were placed in transparent portion cup trays (Bioserv #9040) then placed in a larger container (Sterilite[®] #1859 69L) with 5 cm standing water in the bottom for added humidity. Emergence of phorid flies was checked daily by gently slapping the top of the tray to dislodge any entangled flies from the chif-

fon and to induce activity. Trays containing cups were held to the light and viewed from below to detect emerged flies. Upon detection of emerged flies, the date of emergence and sex of the phorid fly was recorded.

Data Analysis

Due to differing number of ants exposed at d 6 and d 10, the number of ants exposed to each treatment, number of decapitated heads, and number of emerged flies were standardized into proportions. Data were analyzed using factorial analysis of variance (ANOVA), and multiple comparisons made using a Fisher's LSD test (SAS 2008).

RESULTS AND DISCUSSION

The mean proportion of exposed ants that were decapitated varied across the time and treatment interaction (Fig. 1, P value from AOV = 0.0045). There were no significant differences in the proportions of decapitated ants among any of the d 6 treatments: pyriproxyfen 6, methoprene 6, and control 6. Furthermore, no significant differences were observed in the proportion of decapitated ant heads between the methoprene treatments at the 2 time intervals or between the 2 pyriproxyfen treatments. Ants exposed to methoprene at d 10 had the lowest proportion of heads decapitated (0.18), relative to the pyriproxyfen at d 10 (0.25) and control at d 10 (0.28).

Because the third instar decapitates the ant's head prior to pupation, decapitation was deemed to be the observation of importance, rather than the formation of the puparium. Decapitation is a visual indicator of the status of the larva inside the ant, having survived to third instar. Further-

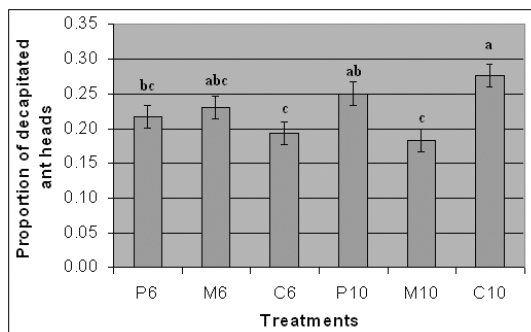


Fig. 1. Mean proportion (\pm SE) of decapitated heads from ants exposed to methoprene and pyriproxyfen at d 6 and 10. Bars with the same letter label are not significantly different ($P > 0.05$). Treatments are abbreviated as follows (P6, pyriproxyfen at d 6; M6, methoprene at d 6; C6, control at d 6; P10, pyriproxyfen at d 10; M10, methoprene at d 10; C10, control at d 10).

more, if the 2 treatments were to have had an impact on decapitation, it suggests an impact of the IGRs on previous instars. The difference between the most disparate treatments (Fig. 1), suggests little biological difference, even though there are statistical differences among treatments.

Both methoprene and pyriproxyfen treatments significantly reduced fly emergence from decapitated heads relative to controls (Fig. 2, P value from AOV < 0.0001). There were no significant differences in emergence between the times of exposure or between the 2 IGR treatments. It is worth noting that the methoprene treatment at d 10 had no flies emerge, and the pyriproxyfen treatment at d 10 only had one fly emerge (0.002) from all 4 replications. Emergence from IGR treatments were significantly less than that from controls with 19 flies emerging from the pyriproxyfen treatment (0.032), and 13 flies from the methoprene treatment (0.012), versus 190 flies from the control (0.256).

Methoprene and pyriproxyfen are not only used in imported fire ant suppression, but also used extensively to control dipteran pests. These products induce mortality of the last instar, a reduction in emerging adults, and an inhibition of development (Bull & Meola 1993; Arshad et al. 2008; Sihuincha et al. 2005; Mascari 2008). Although in this experiment the fly larvae were exposed to IGRs via cutaneous absorption by the ant, similar effects were seen.

There were no significant differences in the mean proportion of flies emerged from the total number of exposed ants between the times of exposure or between the 2 IGRs. Overall, the proportions of emerged flies from ants exposed via filter paper were significantly greater in the controls than the treatment groups ($P < 0.05$). These data suggest methoprene and pyriproxyfen reduce emergence of *P. tricuspsis*. The pattern of significance between the controls and the IGR treatments were similar to those for the proportion of

decapitated heads resulting in emerged flies (Fig. 2).

Fully-formed fly puparia were observed during the process of separating and measuring decapitated ant heads. Feeding-stage larval parasitoids are more sensitive to IGR exposure, due to the consumption of host tissue and hemolymph (Beckage 1985). Because the third instar phorid fly consumes the head contents of the fire ant prior to pupation (Porter 1998; Consoli et al. 2001), this suggests that the impact on development occurred after decapitation of the ant's head.

A common impact on parasitoids exposed to IGRs is a change in the sex ratio (Ascerno et al. 1980; Beckage 1985; Suma et al. 2009). However, the sex of the phorid fly is determined by the size of the ant's head capsule in which it develops, with females emerging from larger ant heads (Morrison et al. 1999). The overlap of ant head size between the phorid fly sexes was within the documented range for *S. invicta* females (1.03 ± 0.08 mm) and males (0.87 ± 0.09 mm) (Morrison et al. 1999). Thus, no discernible differences were found in the sex allocation of *P. tricuspsis* across treatments.

CONCLUSION

The results show that methoprene and pyriproxyfen can hinder *P. tricuspsis* development when parasitized ants are exposed to an IGR-treated substrate in the laboratory. Additional studies are needed to determine if these IGRs have any effect on parasitoid development and survive under field conditions. Data must also be taken to determine if this level of incompatibility of biological and chemical controls interferes with the ultimate goal of sustainable imported fire ant suppression. Formulated baits need to be evaluated in a manner that simulates true field rate exposure and that incorporates feeding behaviors of the ants. Presence of other caste members would incorporate the trophallactic movement of the IGRs throughout the colony.

As additional *Pseudacteon* spp. are approved for release, they should be evaluated based on their compatibility with IGR baits. Because size and development rate vary across *Pseudacteon* spp. (Folgarait et al. 2002), IGRs may be metabolized differently. Additionally, other IGR baits used for imported fire ant control (e.g., fenoxycarb), should be evaluated because their effects on *Pseudacteon* spp. development and emergence are unknown.

Finally, the influence of IGR baits on the F_1 progeny of flies that emerged from ants exposed to IGRs should be evaluated. These sublethal effects of insecticides are profound in other systems (Vinson 1974; Ascerno et al. 1980; Bellés & Ventura 1981; Beckage 1985; Liu & Stansly 1997;

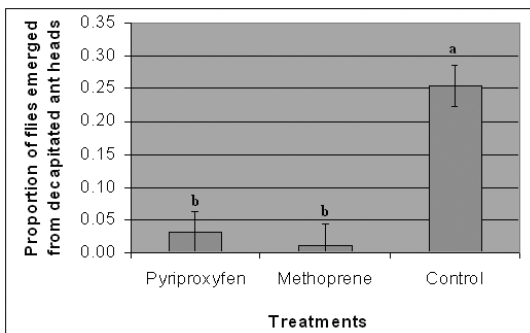


Fig. 2. Mean proportion (\pm SE) of emerged *P. tricuspsis* adults from decapitated ant heads. Bars with the same letter label are not significantly different ($P > 0.05$).

Suma et al. 2009). Knowledge of the impact of IGRs on *Pseudacteon* spp. and when this impact occurs may be an important factor in determining release sites, the *Pseudacteon* spp. to release, and the timing of IGR bait applications for use in managing *S. invicta*.

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