

Rearing the Decapitating Fly *Pseudacteon tricuspis* (Diptera: Phoridae) in Imported Fire Ants (Hymenoptera: Formicidae) from the United States

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ABSTRACT The South American phorid fly *Pseudacteon tricuspis* Borgmeier was imported into quarantine facilities in Gainesville, FL, to study its life history and determine if it could be reared on imported fire ant workers from North America. We found that this fly developed successfully on *Solenopsis invicta* Buren workers from Florida and hybrid *Solenopsis richteri* Forel × *S. invicta* fire ants from Mississippi. It also was reared on *S. richteri* and *S. invicta* fire ants from Argentina. This fly, like its congener *Pseudacteon litoralis* Borgmeier, had the peculiar habit of decapitating its living host and using the ant's empty head capsule as its pupal case. We were able to rear this fly through 1 complete generation in the laboratory, indicating that mass rearing for inoculative releases in the United States may be possible.

KEY WORDS *Solenopsis invicta*, *Solenopsis richteri*, biological control, Argentina, parasite

WHEN THE IMPORTED fire ants *Solenopsis invicta* Buren and *Solenopsis richteri* Forel were introduced into the United States, almost all of their natural enemies were left behind in South America (Jouvenaz 1983). Fire ant densities are about 5 times higher in the United States than they are in South America (Porter et al. 1992, Porter et al. 1997). Although the cause of this difference has not been established, the relative absence of natural enemies in North America is a likely explanation because obvious factors such as climate, habitat, season, and the frequency of polygyny do not account for intercontinental differences in fire ant populations (Porter et al. 1997).

Phorid flies in the genus *Pseudacteon* are a promising possibility for fire ant biological control because (1) they appear to be highly specific (Porter et al. 1995a), (2) they are broadly distributed across climate, habitat, and season (Borgmeier and Prado 1975, Pesquero et al. 1996), and (3) they have had sufficient impact on fire ant populations to have caused the evolution of phorid-specific defense behaviors, the most important of which is the termination of foraging activity whenever phorid flies are present (Feener and Brown 1992, Orr et al. 1995, Porter et al. 1995c).

The life cycle of immature *Pseudacteon* flies is known only for *P. litoralis* Borgmeier (Pesquero et al. 1995, Porter et al. 1995b). The maggot of this species develops in the head of a fire ant worker. It has the unusual habit of decapitating its living host and consuming everything inside the head. The maggot then pupates inside the empty head capsule. Unexposed portions of the phorid pupar-

ium remain unsclerotized, but the 1st 3 segments of the puparium are compressed to form a distinctive sclerotized plate that precisely fills the oral cavity of the ant head capsule.

Our first objective was to determine if the unusual and highly specialized life cycle found in *P. litoralis* occurs in other members of the genus *Pseudacteon*. We also were very interested in determining if *Pseudacteon* flies which attack fire ants in South America would oviposit and develop successfully on imported fire ants in the United States. Finally, we hoped to develop a way of rearing these flies under laboratory conditions. The ability to rear these short-lived parasites is important because it would greatly facilitate further studies of specificity (Porter et al. 1995a) and possible inoculative releases in the United States.

Materials and Methods

About 80 *S. richteri* workers were collected during the first week of April 1995 after having been attacked by ovipositing *Pseudacteon tricuspis* Borgmeier flies in Las Flores, Buenos Aires Province, Argentina. These ants were placed into screw-cap plastic vials (12 by 80 mm) with a wet cotton plug at the bottom. We also collected 11 female and 26 male *P. tricuspis* flies in Las Flores on the afternoon of 5 April using double-chambered Allen aspirators (BioQuip, Gardena, CA). These flies were transferred to plastic vials (17 by 95 mm) with moist dental plaster plugs in the bottom and a strip of loose tissue paper for the flies to run on. These vials were capped with lids vented

with fine-mesh fabric to reduce condensation and the probability that the flies would become entangled in the condensed water. Vials with adults were slid into predrilled foam blocks for insulation and placed in a light-proof bag to inhibit flight activity. Adult flies and the parasitized workers were hand-carried to Gainesville, FL, on the morning of 7 April. About 90% of the adult flies and 75% of the workers survived the trip. All work in Gainesville was conducted in quarantine facilities operated by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

We released the flies into 3 vented flight boxes (13 by 19 by 21 cm, 30 by 26 by 26 cm, and 74 by 47 by 46 cm; length by width by height). The 2 smaller boxes were constructed of clear plastic and the larger box had a clear plastic top. In the 2 larger boxes, we placed 4 shallow trays with *S. invicta* from Reconquista, Argentina; an *S. invicta* × *S. richteri* hybrid from Tupelo, MS; and 2 trays with *S. invicta* from Gainesville, FL. The trays were coated with Fluon to confine the ants. Gas chromatography was used to identify the hybrid ants (Vander Meer et al. 1985). Each tray contained 50–100 mostly medium and large workers. Only *S. invicta* workers were placed in the smallest flight box. The boxes were placed near a window under fluorescent room lighting.

Workers attacked by flies in the flight boxes were combined in groups of 4–10 with several dozen minor worker adults and brood from their mother colony. These extra individuals were added to create small functioning fragment colonies in the hope that this would improve the success of developing parasites. Only minor workers and minor worker brood were added so we could distinguish them from the larger workers which had been attacked. The *S. richteri* workers attacked in Argentina were combined in groups of 2–6 together with a dozen or so minor worker pupae and larvae collected from *S. invicta* colonies around Gainesville, FL. We do not know how fully integrated these composite colonies were, but the *S. invicta* brood was adopted, and no signs of fighting were observed when they eclosed as adults.

Fragment colonies were placed in small foraging trays (22 by 7 cm) with petri dish nests (55 mm), or they were placed into test tube nests (100 by 13 mm) with a cotton/water plug at one end and a foraging tube coupled to the other end. This foraging tube (70 mm) was coupled to the nest tube with a piece of Tygon tubing and plugged at the other end by a wad of cotton. All test ants were provided daily with frozen crickets and tissue paper balls soaked in 1 M sugar water. Half of the parasitized ants were kept at $24.4 \pm 0.4^\circ\text{C}$ and the other half at $30.1 \pm 0.4^\circ\text{C}$. The ants at 24°C were kept under variable room lighting; the ants at 30°C were kept in darkness except during feeding and handling. Fly pupae reared at 30°C were kept in small glass vials with a cotton/water plug in the bottom and a cotton plug in the top. To avoid ini-

tial problems with mold, fly pupae at 24°C were put inside small gel capsules which were placed in a tightly sealed box with a saturated NaCl solution that maintained humidity at 75% (Winston and Bates 1960).

All *P. tricuspidis* flies used in this study matched Borgmeier's (1925) original drawing of his type specimen from Argentina. Voucher specimens of flies and ants have been deposited in the Departamento Científico de Entomología, Museo de la Plata, Universidad Nacional de la Plata, Argentina; the Museu de Zoologia, Universidade de São Paulo, Brazil; and the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Service, Division of Plant Industry, Gainesville, FL. Means in this paper are shown \pm 1 SD.

Results and Discussion

We released the adult flies from Argentina into quarantine flight boxes in Gainesville at 1100 hours (EDT) on 7 April 1995. The flies flew around the corners of the boxes, but no ovipositional activity was observed for ≈ 2 h. Oviposition began around 1300 hours in the large flight box and continued consistently until 1900 hours when the lights were turned off and the flies stopped flying. Most of the attacks occurred on hybrid *S. richteri* × *S. invicta* workers from Mississippi and the *S. invicta* workers from Argentina. Some attacks also were observed on 1 *S. invicta* colony from the United States, but not on a second *S. invicta* colony. It is unclear whether these differences represented a preference for different host species, differences in colony attractiveness, or simply a strong preference for the same tray in which other phorids were active.

Both male and female flies hovered a few millimeters above the fire ant workers. The males were usually distinguishable because they are slightly smaller than the females and they tracked the movements of ovipositing females rather than fire ant workers. Over the course of the afternoon, we observed several dozen mating attempts. Both males and females appeared to be involved in multiple attempts. Most attempts lasted < 1 s and broke up when the pair fell to the bottom of the tray. On several occasions the mating pair continued in contact for 2–3 s after hitting the tray. This is the first report of mating attempts occurring in the laboratory. Previous authors have reported that both males and females are attracted to fire ants in the field (Williams 1980, Feener and Brown 1992, Porter et al. 1995c) and that mating occurs while females are searching for suitable hosts (Feener 1987).

On the 1st day, some oviposition activity was observed in the middle-sized box but none in the small box. On the second day, all of the phorids in the middle-sized box were dead. A few flies in the large box were still alive, but they were not able

to fly. One lone female in the small-sized box attacked *S. invicta* workers for ≈ 3 h on the morning of 8 April.

The developmental histories of *P. tricuspis* and *P. litoralis* are very similar (Porter et al. 1995b). In particular, both species decapitated their host and pupated within the empty worker head capsule. We found that *P. tricuspis* required 20 ± 5 d to hatch and complete larval development, and another 19 ± 2 d to complete the pupal stage for a total developmental time of 39 ± 4 d ($n = 12$, $\approx 24^\circ\text{C}$). This compares to 23, 24, and 47 d, respectively, for *P. litoralis* (Porter et al. 1995b). Just prior to pupation, *P. tricuspis* apparently releases an enzyme or hormone which causes the degeneration of the cuticular membranes that connect the ant head and the 1st pair of legs to the thorax. Sometimes even the other legs and the petiole were affected. Host ants were found decapitated 72% of the time (23/32); the heads of the remaining 28% were usually loose and may have fallen off later.

The maggots consumed all of the tissue in the head of its host, then cut away the maxillo-labial plate and generally one or both mandibles. Upon pupation, the 1st 3 segments of the maggot compressed and sclerotized to form a specialized cap that precisely filled the oral cavity of the ant head. Decapitated ant heads containing phorid puparium were quickly removed from the ant nest by nest-mates and discarded along with other dead workers. The puparium and 3rd instar of *P. tricuspis* were very similar to those previously described for *P. litoralis* (Porter et al. 1995b). An additional observation is that 2 respiratory horns (Disney 1994) were extended out of *P. tricuspis* puparia several days after pupariation. These horns were whisker-like in appearance and about half the length of the cap. They were usually linear, slightly segmented, and had the same red-brown color as the cap. They originated from an unsclerotized area of the puparium just below the sclerotized cap and extended diagonally up and out of the ant head capsule through lateral spaces previously occupied by the ant mandibles. The mean head width of fire ant workers with *P. tricuspis* puparia was 1.16 ± 0.11 mm ($n = 32$). Six male flies emerged from significantly smaller heads than did 4 females (1.15 ± 0.05 versus 1.27 ± 0.06 mm, $t = 3.33$, $df = 8$, $P = 0.012$). This suggests either that females require larger hosts to complete development, or that sex determination in these flies is somehow dependent on the size of their host.

We found parasitic flies in 11–17% of the workers which we observed being attacked. Percentages were similar for the different species of ants. This compares with 35% oviposition success for *P. litoralis* (Porter et al. 1995b). The success rate of *P. tricuspis* larvae developing into puparia was $\approx 70\%$, and the success rate for puparia developing into adults was $\approx 45\%$. We reared fewer puparia at 30°C than at 24°C (2/9 versus 10/18, respectively), but

the difference was not significant (Fisher's Exact $P = 0.22$). About 55% of the maggots pupated without removing both mandibles of their host. Only 20% of these puparia emerged as adults compared with 75% of those that had removed both mandibles (3/15 versus 9/12; Fisher's Exact $P = 0.007$). Overall, we were able to rear adult flies from $\approx 30\%$ of the parasitized workers compared with the 10% reported by Porter et al. (1995b) for *P. litoralis*. Most adults appeared to emerge in the mornings as has been reported for *Drosophila* (Brett 1955).

Adult flies were reared from all 4 types of fire ants tested—*S. invicta* from Argentina (2 flies), *S. richteri* from Argentina (2 flies), *S. invicta* from Florida (2 flies), and the hybrid from Mississippi (6 flies). The fact that flies developed successfully in several species of fire ants is not surprising. *Pseudacteon* species are already known to attack >1 species of fire ant in the *saevissima* complex (Williams 1980, Porter et al. 1995a). Tests in South America indicate that *Pseudacteon* flies will not attack ants in other genera (Porter et al. 1995a), but it is not known whether North American fire ants in the *geminata* complex will be susceptible to attacks from South American *Pseudacteon* flies. We do know, however, that several North American *Pseudacteon* species attack only North American fire ants in the *geminata* complex (Disney 1994).

After emergence, 2nd-generation flies were given access to honey water, fruit juices, plant sap, aphid honeydew, and several kinds of flowers in the flight boxes. However, none of the flies appeared to be attracted to these food sources. To extend the life of flies without mates or between observation periods, we placed them in cotton-stoppered glass vials inside a humid box that was covered by a dark bag, then placed in a 10°C cooler. Each day, the flies were removed from the cooler for several hours for observations or to allow a little activity at normal temperatures. To provide food and water for the flies while they were in the vials, we placed a very thin streak of honey on a strip of laboratory tissue (5 by 25 mm). This tissue was folded in half so that the honey was on the inside, then moistened sufficiently to plaster it to the inside of each vial. This method was used so that the flies would not become entangled in the moisture. Under the microscope, we were able to observe several occasions when the flies clearly lapped up liquid from the wet tissue, especially after periods of activity in the flight boxes. It is not known if they were primarily attracted to the dilute honey or simply the moisture. By using the dark bag and the cool temperatures, we were able to keep the flies alive for 3–4 d before they first stopped flying and died.

When we placed 2nd-generation males and females together in the flight boxes, they generally ignored potential mates and flew around in the corners of the box. Occasionally, one female or 1 male would hover over the ants for 10–20 min.

Several times, females even attacked a few workers, but males were not attracted at the same time. On 18 May, we placed 3 males and 3 females together in the large flight box. After ≈ 3 h of mostly flying around the corners, we raised a tray (22 by 15 by 5 cm) with the hybrid ants from Mississippi to the top of the box and trapped several flies in the tray along with the ants. Within 5 min, the flies began hovering over the ants and ignoring movement of the tray. We were then able to trap the remaining flies in the tray in the same manner, and they too joined in hovering over the ants. Once the flies had found the ants, all 6 flies continued hovering over the ants even though the tray was lowered to the bottom of the box. While the 3 females were striking the ants, the 3 males frequently hit and apparently mated with the females. This activity continued for ≈ 2 h after which 3 of the flies either died naturally or were killed by the ants. The remaining 3 flies were recovered and placed in the cooler as described above. The next day, none of them would fly again and they died.

All hybrid workers exposed to attacks of 2nd-generation flies were placed in small trays at 24°C along with minor worker nestmates and brood as previously described. We eventually recovered 4 3rd-generation parasites (2 larvae and 2 puparia) from these workers.

In summary, this study showed that the phorid fly, *P. tricuspis*, has the same life cycle as its congener *P. litoralis*. We also found that *P. tricuspis* can successfully oviposit and develop in the North American fire ants *S. invicta* and the *S. invicta* X *S. richteri* hybrid. We also were able to rear this parasite through 1 complete generation, including mating and oviposition under laboratory conditions. This accomplishment is important because it suggests that mass rearing of this parasite for inoculative release may be possible with improved rearing techniques.

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