Confinement-Tray Background Color Affects Parasitism Rates of Attacking *Pseudacteon curvatus* (Diptera: Phoridae) in a Laboratory Rearing System[1]

Larry G. Thead[2] and Douglas A. Streett

USDA, ARS Biological Control of Pests Research Unit, P. O. Box 67, Stoneville, Mississippi 38776 USA

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Abstract Distribution of the phorid fly (*Pseudacteon curvatus* Borgmeier), a natural parasitoid of imported fire ants (*Solenopsis* spp.), was tested in a laboratory rearing system to evaluate differences in phorid fly production based on tray background color. Significantly ($P = 0.02$) higher rates of parasitism were observed for ants confined within a tray with darker background colors (olive drab, brown or ruddy brown) than for ants confined within a brighter white background tray. This study identifies a factor that may be influencing production levels in a phorid rearing system.

Key words: phorid, rearing, imported fire ant, *Solenopsis invicta* X *richteri*, visual cues

Successful laboratory rearing of *Pseudacteon* spp. (phorid) flies that are parasitoids of imported fire ants (*Solenopsis* spp.) uses large attack boxes as described by Vogt et al. (2003). A series of white plastic trays within each attack box contains live host ants which are exposed to phorid attack for 3-4 d. The ants are then removed and held for parasitoid development. During parasitoid presence, host ants confined inside each tray are induced to trail back and forth within each tray by providing brood for them to carry and alternately raising and lowering paired, inverted cups under which the ants seek shelter. This system reduces ant "freezing" and "clumping" behaviors and constantly exposes them for attack by the phorid adults. Specific climatic conditions (26° to 29°C, 80-90% RH) are maintained within the attack box to minimize fly and ant mortality and to maximize attack rates. Other external factors, including light intensity, affect the distribution of attacking phorids in the attack box (Vogt 2002). Controlling these factors helps maximize fly distribution.

Porter (2000), Porter and Alonso (1999), Porter and Briano (2000), Folgarait et al. (2002) note that *Pseudacteon* flies are host specific, preferentially attacking one species of ant and size of workers. It is postulated that olfactory cues are significant in flies locating the proper host from a distance and, as the flies hover closer to the host, visual cues are likely critical for host parasitism (Gilbert and Morrison 1997, Orr

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[2]Corresponding author present address: Mississippi Department of Agriculture and Commerce, P. O. Box 5207, Mississippi State, MS 39762, USA; *E-mail address*: LarryT@mdac.state.ms.us.

et al. 1997, Porter 1998, Porter and Alonso 1999, Morrison and King 2004). If visual cues play a role in host attraction, the brightness of a white tray containing the exposed ants, may not be the optimal for the flies to locate and parasitize ants. In nature, an attacking fly rarely encounters a bright white background. White trays are routinely used inside the attack box and in the field to survey for fly establishment in areas where the flies have been released. Attacking behavior of phorid flies is easier to monitor against a white background. These researchers and others (i.e., pers. comm.) noted that objects such as the disturbed ant mound or ants placed on rocks located near a disturbed ant mound attract more flies than the white trays containing ants. Juusola and French (1997) noted that background light level strongly affects the resolution of moving objects by a *Calliphora* spp. fly. The additional light reflected from a white background tray could interfere with the flies' visual cues explaining why fewer flies are attracted to white background trays.

In this study, experiments were conducted to examine the effect of confinement-tray background color on the actual rates of parasitism. The purpose of this work was to optimize use of rearing space and ultimately increase the number of flies reared in the system.

Materials and Methods

The attack box described by Vogt et al. (2003) for rearing *Pseudacteon curvatus* Borgmeier at the USDA-ARS, Biological Control and Mass Rearing Research Unit, MS State, MS, was used for the experiments. The configuration of the attack box allowed for 13 white plastic trays (27.5 cm W × 42 cm L × 12 cm H) that were cleaned thoroughly prior to painting. The dark backgrounds encountered by *P. curvatus* in nature were simulated with three colors of Krylon® aerosol paint (Krylon Products Group, The Sherwin Williams Co., Cleveland, OH). Each of the aerosol paint colors, camouflage ultra flat olive drab # 8143, camouflage ultra flat brown # 8142 and primer ruddy brown # 1317 were applied to three trays each. The entire inside surfaces of the trays were painted except for a 5 cm edge at the top perimeter of each tray and allowed to air dry for a minimum of 3 wks. Three trays representing the control treatment and one tray for maintaining phorid rearing were not painted. Prior to use, all trays were washed with detergent and water to remove any residue and coated with Fluon® (Asahi Glass Ltd., Chadds Ford, PA) along the upper 5 cm edge to prevent ant escape.

Hybrid fire ants (*Solenopsis invicta* × *richteri*) were collected from Oktibbeha Co. and Noxubee Co., MS, Nov. and Dec. 2003, using the collecting and preparation procedures described by Vogt et al. (2003). Ants and brood were floated from the soil (Banks et al.1981), then workers were passed through a 20-mesh sieve to separate smaller workers that are preferred by *P. curvatus* (Morrison and Gilbert 1998). Colonies were maintained in an environmentally controlled room at ~27°C, 60% RH and a 12:12 (L:D) h photoperiod with water, sugar water, and crickets (*Acheta domestica* L.) *ad lib* for 2-3 wks prior to use in experiments. Ants from a single colony were used for each experiment. Approximately 1.2 g of workers (about 1500 ants) collected from a single colony along with approximately 1 g of brood were placed into each of the 13 trays at the beginning of each experiment. The ants and brood were held inside the attack box for 3-4 d where they were exposed to emerging *P. curvatus* [Las Flores, Argentina biotype (Porter 2000)]. The numbers of emerging *P. curvatus* varied for each experiment, resulting in varying levels of parasitism for each experiment. This factor was not controlled because the experiments were conducted within an attack box used for routine rearing purposes.

A randomized complete block design using 12 trays was used for each experiment with the four tray colors (olive drab, brown, ruddy brown, and white) randomly assigned to each block. Blocks corresponded to proximity to the location of the emerging flies with one block (4 trays) next to the emerging flies, another (4 trays) in a central position and the final four trays farthest from the location of the emerging flies. The tray for maintaining phorid rearing was located adjacent to the sealed side of the fly resting box.

After exposure to attacking flies for 3-4 d, the ants were removed from the attack box. Dead ants were discarded, brood were removed, and the remaining live ants from each tray were placed inside individual sealed, fluon-coated and ventilated Rubbermaid® Servin' Saver® (Newell Rubbermaid Inc. Freeport, IL) containers (19 cm L × 11.5 cm W × 5 cm H). A covered plastic Petri dish (60 × 15 mm) containing cured castone dental stone (Dentsply Trubyte, York, PA) and a watered sugar-impregnated Techwipe® (Horizon Industries, Tyler, TX) were placed inside each container. These individual containers were held in an environmentally controlled room at ~27°C, 60% RH, and a 12:12 (L:D) h photoperiod. Three times per week for approximately 34 d, dead ants were collected and evaluated for parasitism. The numbers of ants parasitized per tray were recorded. The experiment was repeated three times using a different ant colony and a different tray randomization each time. Data for each colored tray within each block location were subjected to PROC GLM (SAS Institute1990) to test for the effects of background tray color and distance from point of emergence (i.e., block effect). Significant effects were subjected to GLM least squares means test.

Results and Discussion

Analysis indicated that the inability to control the numbers of attacking *P. curvatus* for each experiment resulted in a significant difference among experiments ($F = 7.49$; $df = 2, 28$; $P = 0.0025$). Adjustment for the experimental effect required conversion of data using the equation: % parasitized ants per colored tray = (number of parasitized ants per colored tray ÷ number of parasitized ants per experiment) × 100. A significant block effect ($F = 4.15$; $df = 2, 24$; $P = 0.028$) was observed with significantly higher levels of parasitism in the four trays nearest the phorid release box. The four trays nearest the phorid release box represented 48.1% of the parasitized ants for all experiments. The middle four trays and farthest four trays from the phorid release box represented 24.3% and 27.6%, respectively, of the parasitized ants for all experiments. The block color interaction was not significant ($F = 2.06$; $df = 6, 24$; $P = 0.097$).

The background color of the trays had a significant effect ($F = 3.81$; $df = 3, 24$; $P = 0.023$) on the level of parasitism. There were no differences between the numbers of ants parasitized in the dark background colored trays, but all had significantly more parasitized ants within them than the white background trays (Table 1). Overall, flies attracted to the dark background trays parasitized approximately 2.6 fold more ants than in the white background trays. When the parasitism rates by flies attracted to individual colors were examined, the olive drab, ruddy brown and brown showed a 2.9, 2.8 and 2.1 fold increase, respectively, in the parasitism rate versus the parasitism rate in the white background trays. A fly preference for the darker background colored trays as opposed to the brightness of the white background trays is demon-

Table 1. Mean percent-parasitized ants per tray background color

Tray background	Mean % parasitized ± *SE*	*P*
Olive drab	33.31 ± 5.05	<0.0001
Brown	23.25 ± 5.05	0.0001
Ruddy brown	32.09 ± 5.05	<0.0001
White	11.34 ± 5.05*	0.0342

* Significantly different from other treatments (Least Squares Means Statistics).

strated. Inferences can be made that host attraction visual cues, as perceived by *P. curvatus*, are more likely influenced by the darkness of the background rather than the background color.

This study demonstrates how confinement-tray dark background colors may be influencing production of phorids in a mass rearing program. Additional studies are needed to determine if altering the background color of the trays within the attack box would result in an overall increase in rates of parasitism as compared with the current rearing system using the white background trays.

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