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Host preference in *Pseudacteon* phorid flies: response of *P. tricuspis* and *P. curvatus* to black, red and hybrid imported *Solenopsis* fire ants in multiple choice bioassays

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ABSTRACT

Host preferences in both sexes of *Pseudacteon tricuspis* Borgmeier (Jaguariuna biotype) and *Pseudacteon curvatus* Borgmeier (Formosa biotype) and their relative attraction to the imported fire ants (IFA), *Solenopsis invicta* Buren (red IFA), *Solenopsis richteri* Forel (black IFA) and *S. invicta* × *S. richteri* hybrids (hybrid IFA) were investigated in two separate experiments utilizing multiple choice flight bioassays. The results of both experiments clearly showed that both sexes of the Jaguariuna biotype of *P. tricuspis* could distinguish among the three IFA species and demonstrated greater preference for hybrid IFA and red IFA. This conclusion is supported by a variety of data collected on the number of fly visits, attack rate, and hovering duration (Experiment 1), and on the number of trapped flies (Experiment 2), which showed that black IFA is the least preferred of the three species. Similar results were recorded for the Formosan biotype of *P. curvatus*, although the data were not as strongly conclusive. Females of this biotype spent a significantly greater amount of time in hovering mode over red IFA and hybrid IFA compared to black IFA, but the other data were not significant. The red IFA is the natural host of both phorid fly biotypes and our results suggest that both biotypes may have evolved a specialized relationship with red IFA including an ability to discriminate it from related fire ants. These results are discussed in relation to the possible role of fire ant chemicals in mediating host preferences in phorid flies, contributions of male phorid flies to fire ant biocontrol, and the practical implications of the key findings.

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1. Introduction

Two species of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae) were accidentally introduced from South America into southern United States (U.S.) in the past century. The black imported fire ant (black IFA), *Solenopsis richteri* Forel was introduced around 1918 followed by the introduction of the red imported fire ant (red IFA), *Solenopsis invicta* Buren in the early 1930s. Both species are believed to have entered the U.S. through the port of Mobile, Alabama, spreading northward in Alabama and into neighboring states (Wilson, 1958). Hybridization between *S. invicta* and *S. richteri* (producing hybrid IFA) has been documented in Alabama, Mississippi, Georgia, and Tennessee (Vander Meer et al., 1985; Ross et al., 1987a,b; Diffie et al., 1988; Vander Meer and Lofgren, 1988; Shoemaker et al., 1996). These invasive fire ants are now widely distributed throughout the southern U.S. inhabiting more than 320 million acres (Williams et al., 2003) and causing a multitude of problems for humans, domestic animals, and agriculture in the region.

Four species of *Pseudacteon* decapitating phorid flies (Diptera: Phoridae) have been introduced from South America in the past decade as classical biological control agents of imported fire ants in southern U.S. (Feener and Brown, 1992; Orr et al., 1995; Gilbert, 1996; Porter et al., 1995a; Porter and Alonso, 1999; Porter and Gilbert, 2004; Gilbert et al., 2008). The two key species were *Pseudacteon tricuspis* Borgmeier and *Pseudacteon curvatus* Borgmeier (Porter and Gilbert, 2004; Vazquez and Porter, 2005). One of the early successful introductions of phorid flies in southern U.S. involved a biotype of *P. tricuspis* collected from Jaguariuna, Brazil in 1996 on red IFA (Porter and Alonso, 1999; Porter et al., 2004; Pereira and Porter, 2006). This biotype is now well established in several southern states (Pereira and Porter, 2006). In addition, two biotypes of the second species, *P. curvatus* have been released in the region. A biotype from Las Flores, Argentina was released to control populations of black IFA and hybrid IFA in Alabama, Mississippi and Tennessee (Graham et al., 2003; Vogt and Streett, 2003; Parkman et al., 2005). A second biotype from Formosa, Argentina, which was also collected on red IFA, was released on red IFA in Florida, South Carolina and Texas (Vazquez et al., 2004; Davis and Horton, 2005; Gilbert et al., 2008).

Both species of phorid flies (*P. tricuspis* and *P. curvatus*) were shown in pre-release laboratory host range tests to be highly specific to imported fire ants and minimal nontarget effects on native fire ants were predicted (Gilbert and Morrison, 1997; Porter and Alonso, 1999; Vazquez et al., 2004). Field studies also showed that

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the flies were not attracted to native fire ants or ants from other genera (Porter, 1998b; Morrison and Porter, 2005; Vazquez and Porter, 2005), and their highly specialized behavior and life history make them unlikely threats to other arthropods (Porter, 1998a; Porter and Gilbert, 2005).

Matching biotypes of potential biological control agents to target host populations can significantly improve the effectiveness of biological control agents (Van Driesche and Bellows, 1996; Porter and Briano, 2000; Porter and Gilbert, 2005). However, very little is known about the host preferences of the different species and biotypes of phorid flies. In perhaps the only published study on parasitoid-host matching in phorid flies, Porter and Briano (2000) showed that the Las Flores, Argentina biotype of *P. curvatus* preferred black IFA and hybrid IFA over red IFA. However, this study was carried out using no-choice and pair-wise choice bioassays making it impossible to compare attraction of the flies to the three imported fire ant species/forms in a single test.

Parasitism has been used as the ultimate parameter for determining host specificity and preference in phorid flies (Porter and Briano, 2000; Vazquez et al., 2004). However, studies have showed that parasitism rates of phorid flies on ants are consistently less that 5% in the field (Feener, 1981, 1988; Morrison et al., 1997; Morrison and Porter, 2005), suggesting that the direct effect of ant mortality in the field due to phorid flies is very low. On the contrary, pre-parasitization behaviors of phorid flies (e.g., hovering and attack attempts) resulted in a 50% decrease in food retrieval by fire ants (Morrison, 1999), suggesting that these behaviors may play very important roles in the effectiveness of phorid flies as ant biological control agents.

Aside from their primary role in mating with females, little is known about the contributions of male phorid flies to ant biological control. In *P. tricuspis*, both sexes are attracted to fire ants and mating occurs while females are searching for ant workers to attack (Porter, 1998a). Male and female *P. tricuspis* also display similar hovering behavior over fire ants (Porter, 1998a). Furthermore, response of both sexes of *P. tricuspis* to odor of live *S. invicta* workers and their body extracts have been reported (Chen and Fadamiro, 2007). These findings suggest that *P. tricuspis* males may be providing more contributions than previously thought to the overall biological control effects of phorid flies in the field. However, in *P. curvatus*, mating occurs elsewhere and males are unlikely to contribute much to biological control of fire ants since they are not attracted to fire ant workers (Wuellner et al., 2002).

The main objective of this study was to determine host preferences in both sexes of *P. tricuspis* (Jaguariuna biotype) and *P. curvatus* (Formosa biotype) by testing their relative attraction to the three imported fire ant species/forms in multiple choice flight bioassays. Males of *P. tricuspis* were tested because they are attracted to fire ants (Porter, 1998a). Thus, we hypothesized that *P. tricuspis* males will demonstrate similar host preferences as conspecific females. However, *P. curvatus* males were tested simply for comparison and to confirm a previous report which suggested that they are not attracted to fire ants (Wuellner et al., 2002). Host preference was evaluated using pre-parasitization behavioral parameters such as number of phorid flies attracted to ants, number of fly visits to ants, number of attacks on ants per minute (attack rate), and the amount of time spent by flies in hovering mode over ants (hovering duration).

2. Materials and methods

2.1. Imported fire ants

Colonies of black IFA and hybrid IFA were collected in northern Alabama and western Tennessee (USA) in spring 2008. Red IFA colonies were collected on the campus of Auburn University, Auburn, Alabama. Ant workers were collected by transferring about 1 l of soil (containing workers, broods and the queen) from each mound into 1-gallon (3.785-l) Rubbermaid plastic jars coated with Fluon® (ICI, Wilmington, DE) to prevent escape. Workers collected from each colony were maintained in their nest soil in the laboratory at 25 ± 1 °C, 50 ± 10% relative humidity, on a 14:10 h light:dark cycle and fed 10% sucrose solution and crickets. The colonies were maintained for 1–2 months after collection before the tests. Colonies were separated and identified as red IFA, black IFA and hybrid IFA by analysis of worker venom alkaloids and cuticular hydrocarbon profiles using gas chromatography (Vander Meer et al., 1985), as recently described by Fadamiro et al. (2009).

2.2. Phorid flies

Pseudacteon tricuspis (Jaguariuna biotype from Brazil) and *P. curvatus* (Formosa biotype from Argentina) used in this study were reared on the workers of red IFA at the fire ant rearing facility of the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A. as previously described (Porter et al., 1997) and shipped to our laboratory as parasitized fire ant worker heads. Parasitized fire ant worker heads were received in batches and held in a plastic jar (25 × 13 cm) with a lid until emergence. Jars were kept in a growth chamber at 25 ± 1 °C, L:D 14:10 h and 75 ± 5% r. h. Twice daily, newly-emerged flies were removed with an aspirator and species identity and sex were confirmed under a stereomicroscope by using appropriate morphological characters such as the presence of an ovipositor of females (Porter, 1998a; Porter and Pesquero, 2001). One-day old flies which had been provided 10% sucrose solution for 3 h were used for the tests.

2.3. Experiment 1

A methodology similar to the one described by Porter and Briano (2000) was used to evaluate preferences of *P. tricuspis* and *P. curvatus* for the three imported fire ant species/forms (red IFA, black IFA and hybrid IFA). The main difference was that our experiments were multiple choice bioassays compared to the no-choice and dual choice bioassays used by Porter and Briano (2000). The tests were conducted in white plastic trays (42 × 33 × 16 cm) with screened vents and tight-fitting transparent glass lids (Porter, 2000; Porter and Briano, 2000). Four opaque plastic cups (8 cm diameter × 5 cm high; 150 ml) with the inner sides coated with Fluon® (to contain the ants) were placed in the bottom of each tray, one in each of the four corners of the tray. For each trial, workers of red IFA, black IFA and hybrid IFA of similar size were kept separately in each cup (1 species per cup). The fourth cup was left blank and served as negative control. The location of each cup was randomly determined and re-randomized during each replication. Test ants containing 0.25 g of workers (~400) and several broods were placed in each cup. Test ants were first placed in the cup and allowed to acclimate for 30 min before phorid fly release. Twenty one-day old female or male *P. tricuspis* or *P. curvatus* were released through screened vents into the plastic tray without removing the glass lid cover. Separate tests were conducted for each phorid fly species and sex combination. Each test lasted 1 h and the tray was inspected continuously over this duration by a single observer. Tests were conducted between 12:00 and 16:00 h, the time of day for high phorid fly activity (Pesquero et al., 1996). Each phorid fly species and sex combination was replicated 16 times using four colonies of each fire ant species (4 replicates per colony). The number of visits made by flies to each cup (ant treatment) was recorded by visual count (fly visits). "Visiting" flies typically hovered over each cup containing "attractive" ants.