Habitat Associations of Red Imported Fire Ants (*Solenopsis invicta*) and Introduced Populations of *Pseudacteon* spp. Phorid Flies

by

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ABSTRACT

Habitat selection is one of many aspects of red imported fire ants, *Solenopsis invicta* Buren, (RIFA) that has been intensively studied since their initial introduction in the United States. Concurrently, innumerable studies have attempted to identify effective chemical insecticides for RIFA control. More recently, several species of *Pseudacteon* spp. phorid flies (Diptera: Phoridae) have been intensively evaluated to determine their potential utility in biological control of RIFA. These flies belong to a suite of natural enemies of RIFA in their native South American range, and have been selected for release in the United States as biological control agents against RIFA. It is hypothesized that phorid flies, through parasitism and suppression of RIFA foraging, will provide measureable RIFA population suppression as phorid ranges expand and fly densities increase. Relatively little scientific attention has been paid to habitat selection and partitioning by these flies in their introduced range(s) in the US. We assessed RIFA and phorid (*Pseudacteon curvatus* and *Pseudacteon tricuspis*) densities in four central Texas habitats. The habitat types selected represent a continuum of habitat diversity, micro-habitat availability, and plant heterogeneity. Within the habitat types studied, RIFA mound densities were significantly different, but foraging intensity was not significantly different in three of four habitat types. Population densities of *P. curvatus* were determined to be significantly higher in one habitat than all others. Alternatively, *P. tricuspis* densities followed a trend within the surveyed habitats that correlated with RIFA densities observed in this study. These data imply that the successful release, establishment, and range expansion of *P. curvatus* may require a greater degree of critical consideration regarding the ecology

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of each system into which they are released, as opposed to *P. tricuspis.* These implications also suggest that the successful establishment of additional phorid species in the US should take into consideration the habitat matrix and habitat-based expansion corridors available to the flies.

Key Words: *Solenopsis*, *Pseudacteon curvatus*, *Pseudacteon tricuspis*, Invasive Species, Biological Control

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), hereafter referred to as RIFA, is an invasive species that has become established in the United States and elsewhere (Morrison et al. 2004). These ants are native to South America and were discovered in Mobile, AL in the 1930's (Vinson 1997). RIFA populations have since undergone an explosive dispersal and range expansion across the gulf-coast and eastern seaboard states in the continental United States. Their contiguous range now extends from western Texas east to Florida and north to North Carolina. In addition, disjunct populations have become established in Maryland, New Mexico, Arizona, and California. Like many other invasive species of plants and animals, RIFA were liberated from the population regulatory effects of natural enemies in their native range when they arrived in the US. As a result, RIFA pose a significant ecological and economic threat to invaded areas (Lofgren 1986, Porter et al. 1992).

Chemical control measures for suppression of RIFA densities have been the focus of intensive research efforts. Additionally, the past decade has witnessed a large-scale effort to introduce and manipulate populations of natural enemies of RIFA in the US, with the goal of suppressing RIFA populations via biological control (Porter 1998; Gilbert et al. 2008; Oi and Valles 2011). One such suite of natural enemies of RIFA, the *Pseudacteon spp.* phorid flies (Diptera: Phoridae), are known to parasitize workers of the *Solenopsis saevissima* complex of fire ants (including *S. invicta*) throughout their native South American range (Folgarait et al. 2000; Calceterra et al. 2005). Many species of these flies have been released within the United States (Porter 1998; Graham et al. 2003; Vogt and Street 2003; Porter et al. 2004; Allen et al. 2010; and Plowes et al. 2011). Female phorids seek RIFA hosts at mounds and along foraging trails, and oviposit into the thorax of ant workers (Morrison *et al.* 1997).

A significant amount of fundamental research has been conducted with the goal of understanding RIFA/phorid interactions and phorid developmental biology (Feener 1981; Feener and Brown 1992; Folgarait and Gilbert 1999; Morrison 2000; Orr et al. 2003; Consoli et al. 2001; Porter and Pesquero 2001). Development of a successful biological control program which seeks to introduce *Pseudacteon* spp. phorids for suppression of RIFA throughout their non-native range depends upon successful introduction and establishment, and then expansion of phorid range as well as population density increase. In order to successfully choose appropriate release sites, a critical evaluation of the biotic and abiotic factors necessary for phorid establishment is necessary. *Pseudacteon* spp. phorid fly species assemblages are known to partition available niche space based upon selectivity of seasonal and diurnal activity patterns, as well as size of ant hosts (Campiolo et al. 1994; Fowler et al. 1995; Pesquero et al. 1996; Folgarait et al. 2003; Callcott et al. 2011). Relatively little is known regarding habitat utilization and niche partitioning among phorids in their introduced range in the United States.

Pseudacteon tricuspis was released and became established at 5-Eagle Ranch in Burleson County, Texas (30° 38' 15" N; 96° 40' 59" W) in 2002. *Pseudacteon curvatus* was released in the Spring of 2004 at the same site. *Pseudacteon curvatus* is physically smaller than *P. tricuspis* and was selected for its ability to attack polygyne RIFA colonies, which predominate in most of Texas. These colonies are characterized by greater mound densities and a larger proportion of small worker ants relative to monogyne RIFA colonies (Macom and Porter 1996). The first recovery of adult *P. curvatus* occurred during the Spring of 2005, and flies have been collected during all subsequent sampling periods. The presence of RIFA, and establishment of these two phorid species at 5-Eagle Ranch afforded an opportunity to examine the interactions and spatial distributions of ant hosts and introduced parasitoids.

MATERIALS AND METHODS

Experimental Field Sites

This field study was conducted at 5-Eagle Ranch, located in Caldwell, TX (Burleson Co.). The interior area of the ranch is approximately 1,133 ha (2,800-acre) and lies in the East Central Texas Forest ecoregion of south-central Texas (Olson et al. 2001). It is presumed that the ranch first became infested with *S. invicta* in early 1970 (Vinson 1997). Phorid flies, *P. tricuspis* and *P. curvatus* were released at the ranch as part of the USDA-ARS "Area-wide Suppression of Imported Fire Ants in Pastures Project" (Pereira 2003), and are known to have become established by 2003 and 2005, respectively (Vander Meer et al 2007, Gilbert et al 2008).

The entire area encompassed by 5-Eagle ranch was mapped using ESRI ArcGIS® v10.0 (ESRI 2011) software and the interior of the ranch was digitally divided into a grid of 100 m X 100 m cells. All cells were assigned a unique numerical identification code. ESRI ArcGIS® v10.0 (ESRI 2011) software was used to determine the center (centroid) of each selected cell, and the coordinates of those points were generated and stored in a Trimble® GeoXT datalogger .

Sampling sites for this study were then established by first classifying 5-Eagle Ranch based upon its constituent habitat types with ESRI ArcGIS® v10.0 (ESRI 2011) software. Next, four common central Texas habitat types were selected (Hay Pastures, Cattle Pastures, Unmanaged Habitat and full canopy Forest; Fig. 1a,b,c,d respectively). These habitat types represented a continuum of habitat diversity and plant species heterogeneity. The description of each habitat type is as follows:

a) *Hay Pastures-* These pastures consisted of Coastal Bermudagrass *Cynodon dactylon* (L.) Pers. (Bogdan) monoculture, which received one herbicide treatment/year, and were fertilized twice yearly with nitrogen supplements. Cattle were not permitted to graze in these pastures, and hay harvesting occurred 2-3 times annually between May and September.

b) *Cattle Pastures-* These pastures consisted of a mixture of Coastal Bermudagrass *Cynodon dactylon* (L.) Pers. (Bogdan) and a variety of native grasses and shrubs, received one herbicide treatment/year, were fertilized once yearly with nitrogen supplements only, and required occasional (< once/year) shredding. Cattle grazing occurred on a rotational basis.

c) *Unmanaged Habitat-* These pastures were created by removing trees ~25 yrs ago and existed in a transitional ecological state that, if continued to be unmanaged, would presumably revert to the original Post Oak Savanna ecotype. The habitat consisted of grasses and shrubs including Goldenrod

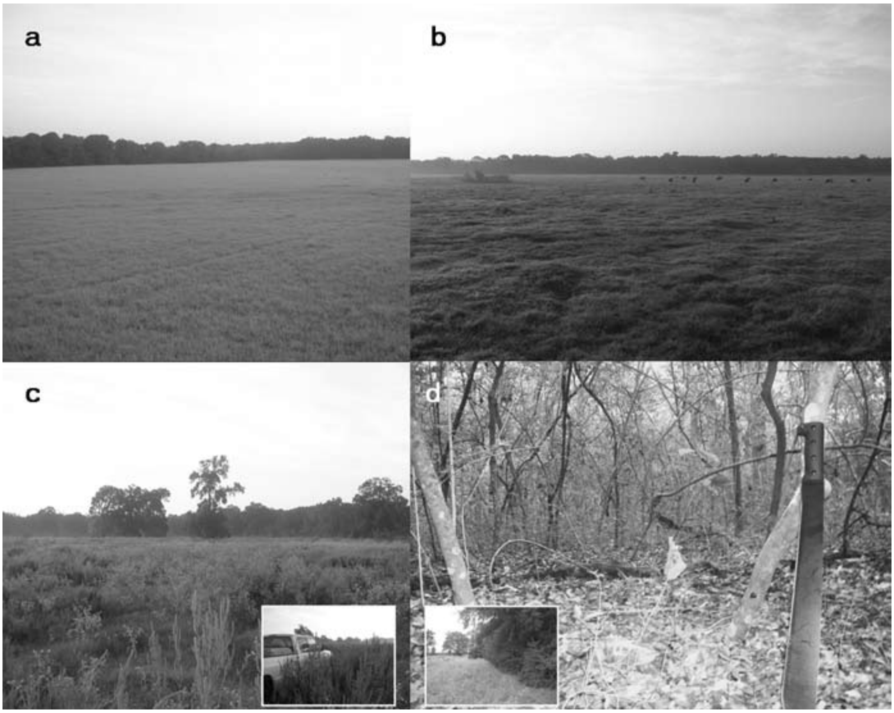

Fig. 1. a) Typical Hay Pasture, b) Cattle Pasture, c) Unmanaged Habitat, and d) Forest at 5-Eagle Ranch. These habitats, from a)-d) respectively, represent a continuum of habitat diversity and plant species heterogeneity.

Solidago sp., Croton *Croton sp.*, Milkweed *Asclepias sp.* and Senna Beans *Senna Obtusifolia* (L.) Irwin & Barneby. Cattle grazing occurred on a rotational basis.

d) *Forest-* Full canopy of oaks *Quercus spp.* with dense Yaupon *Ilex sp.* understory. In addition to cattle, feral hogs, deer and other wildlife had access to much of this habitat, it was not considered part of the managed portion of 5-Eagle ranch and was not manipulated in any way.

Sampling sites were established by selecting four grids of nine contiguous cells within each habitat type (9 cells per habitat types). Where possible, grids were selected from within habitat types in a 3 X 3 block formation. The scale and distance between sampling points in the forest and unmanaged habitats required an alternative configuration of sampling points than hay and cattle pastures. The sampling points were a minimum of 100 m from each other and from alternative habitat types. This is considered a sufficient distance to eliminate potential competition for attraction of phorids between traps and among habitat types (Puckett et al. 2007).

To determine RIFA densities and their utilization of each habitat type, RIFA were monitored by mound counts which involved a direct census of all active RIFA mounds within circular 0.05 ha plots within each selected grid cell. The radius of each circular plot was 12.67 m. Mound counts were accomplished by first locating the center of each cell. Next, a 1.5 m length of rebar was placed at the cell center and a 12.67 m length of cord attached. The cord was pivoted around the center of the cell while four researchers were evenly spaced along its length. Researchers identified, and assessed for activity, all RIFA mounds within the plot until one full revolution was made around the cell center. Only active RIFA mounds were recorded. Additionally, hot-dog lures were used to assess RIFA density and foraging activity within all selected cells. For this assessment, one 0.5 cm slice of Bar-S hot dog was positioned in the center of a 7.62 X 12.7 cm notecard and placed on the ground at the center of the cell. One hot dog slice was placed 5 m to the east and west of the cell center (totaling three hot dog baits per sampling site). After a period of 45 min elapsed, hot dog bait 'hits' (RIFA recruitment to and domination of hot dog bait, to the exclusion of other ant species) were recorded. Comparisons of active mounds and hot dog bait 'hits' were compared statistically via Analysis of Variance (ANOVA) and means were separated using Tukey's Honestly Significant Difference (HSD). The statistical package IBM SPSS v.20.0 (IBM SPSS Inc. 2011) was used to perform these analyses (values significantly different when $P \leq 0.05$).

To assess phorid density and habitat selection differentials, phorid sampling devices known as PTS Traps (Puckett et al. 2007) were deployed at the center of each sampling plot and retrieved after a period of 24 hrs. Traps were returned to the laboratory where phorids were identified to species and counted. Beginning on Sept. 12, 2008, sampling occurred once per week for a total of nine consecutive weeks. Analysis of Variance (ANOVA) was used to analyze the mean number of each individual phorid species per habitat type, and means were separated using Tukey's Honestly Significant Difference (HSD). Students *t*-Test was used to compare the mean number of *P. curvatus* and *P. tricuspis* within similar habitat type. Again, the statistical package IBM SPSS v.20.0 (IBM SPSS Inc. 2011) was used to perform these analyses (values significantly different when $P \leq 0.05$).

RESULTS

RIFA Activity-

Mound Counts- RIFA mounds were significantly less numerous in forest habitat than any other habitat type ($F(3,35) = 20.22$, $P < 0.01$; Fig. 2 and Table 1), and were significantly less abundant in unmanaged habitat than in cattle pastures (Tukey's HSD Post Hoc Analysis, $P < 0.01$; Fig. 2 and Table 1). Mean number of RIFA mounds were not significantly different between hay and cattle pastures (Tukey's HSD Post Hoc Analysis, $P = 0.22$; Fig. 2 and Table 1).

Hot Dog Baits- Hot dog bait hits demonstrated that RIFA were significantly less abundant in forest than in any other habitat types ($F(3,35) = 5.58$, $P < 0.01$; Fig. 3 and Table 1). There were no significant differences in the mean number of foraging RIFA on hot dog baits in the remaining habitat types (Fig. 3 and Table 1).

Phorid Activity

Pseudacteon curvatus- These flies were significantly more abundant on PTS Traps which were deployed in unmanaged habitat than in any other

Table 1. Mean number of active RIFA mounds and hot dog bait 'hits' from within plots in each habitat type.

	RIFA Sampling Methods	
Habitat Type	Mound Counts	Hot Dog Baits
Hay Pasture	12.33 (a,b)	1.55 (a)
Cattle Pasture	16.78 (a)	1.44 (a)
Unmanaged Habitat	7.33 (b)	1.67 (a)
Forest	0.01 (c)	0.11 (b)
P value	< 0.01	< 0.01
F stat	20.22	19.96

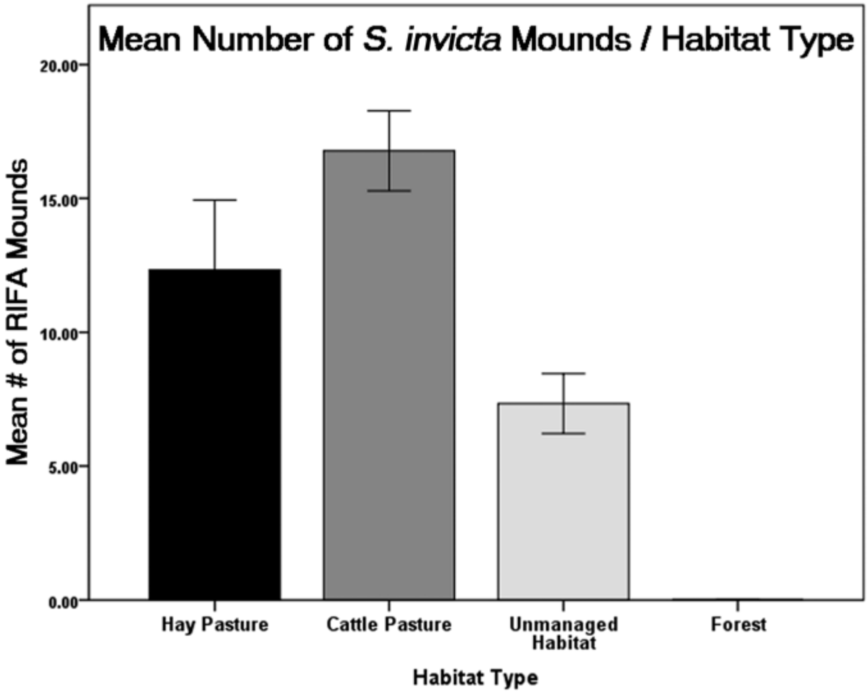

Fig. 2. Mean # of RIFA mounds in all habitat types. Mound densities were significantly higher in cattle pastures than in unmanaged habitat and forest, but were there was no significant mound density difference between cattle and hay pastures. Mound densities were significantly lower in forest than any other habitat type.

habitat type (F (3,323) = 19.96, P < 0.01; Fig. 4 and Table 2). The mean abundance of *P. curvatus* was significantly lower in forest habitat as compared to all other habitat types.

Pseudacteon tricuspis- This phorid fly species was significantly more abundant in cattle pastures as compared to densities observed in unmanaged habitat and forest (F (3;323) = 17.44, P < 0.01; Fig. 4 and Table 2) and their abundance was not significantly different in hay and cattle pastures (Fig. 4 and Table 2). The mean abundance of *P. tricuspis* was significantly lower in forest habitat as compared to all other habitat types.

Pseudacteon curvatus vs P. tricuspis- *P. curvatus* was significantly more abundant than *P. tricuspis* in unmanaged habitat (t (160) = 4.57, P < 0.01; Fig. 4 and Table 2). There was no significant difference in the relative abundance of *P. curvatus* and *P. tricuspis* in hay pastures (t (160) = 0.86, P = 0.39; Fig.

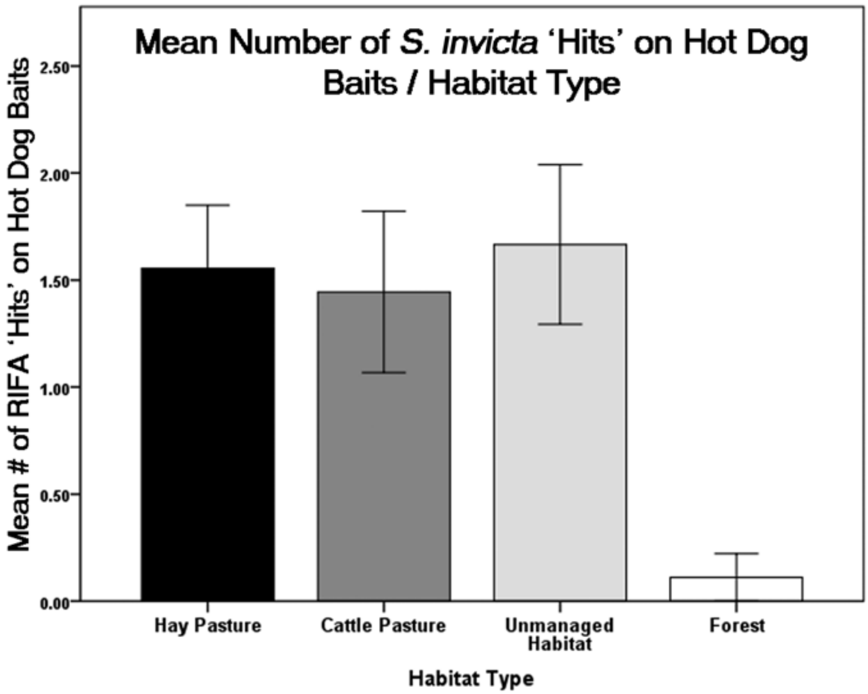

Fig. **3.** Mean # of RIFA 'hits' on hot dog baits in all habitat types. There were no significant differences in RIFA 'hits' on hot dogs in hay pastures, cattle pastures, or unmanaged habitats, and 'hits' in each of these habitat types was significantly greater than in forest plots.

4 and Table 2), cattle pastures (t (160) = 0.35, P = 0.72; Fig. 4 and Table 2) or forest (t (160) = 1.69, P = 0.09; Fig. 4 and Table 2).

DISCUSSION

In this study, RIFA population differentials were related to habitat type. That is, RIFA colonies were significantly more abundant in cattle pastures, and least abundant in unmanaged habitat. With this in mind, a reasonable presumption would be that parasitoids of these ants, such as phorid flies, would likewise be spatially partitioned throughout the available habitat in a similar density composition. In fact, *P. tricuspis* densities followed an identical habitat-specific trend as that of the mean number of RIFA mounds throughout the habitat types surveyed in this study. These flies were most abundant in cattle pastures, followed by hay pastures, unmanaged habitat, and finally forest, where they were almost non-existent. Alternatively, *P.*

Table 2. Mean number of phorids collected in each habitat type.

	Mean # of Phorid Fly Species			
Habitat Type	*P. curvatus*	*P. tricuspis*	*p* value	*t* stat
Hay Pasture	1.71 (b;1)	1.42 (a,b;1)	0.39	0.87
Cattle Pasture	1.99 (b;1)	2.15 (a;1)	0.72	0.36
Unmanaged Habitat	5.76 (a;1)	1.19 (b;2)	< 0.01	4.57
Forest	0.13 (b;1)	0.01 (c;1)	0.09	1.69
P value	< 0.01	< 0.01		
F stat	19.96	17.44		
df	3,323	3,323		

***Means followed by different **numbers** in the same **row** are significantly
Means followed by different **letters** in the same **column** are significantly different (Tukey's HSD, *P* = 0.05)
different (Student's t-Test, *P* = 0.05)

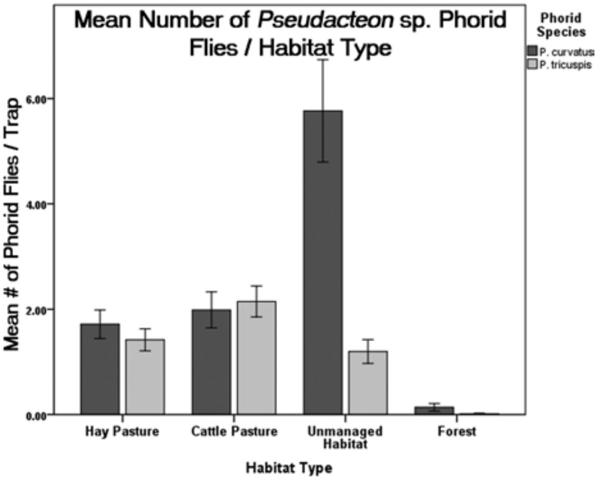

Fig. 4. Relative abundance of *Pseudacteon curvatus* and *Pseudacteon tricuspis* across and within each of four habitat types.

curvatus was significantly more prevalent in unmanaged habitat than the remaining habitat types. Thus, the habitat-specific population density of *P. tricuspis* was more closely correlated to habitat-specific RIFA population density than was that of *P. curvatus*. Very few RIFA or flies of either species were observed in forest habitat.

There were no significant differences observed regarding foraging intensity of RIFA in hay pastures, cattle pastures, and unmanaged habitats; however, foraging in all of these habitat types was significantly more intense than in forest habitat, where negligible RIFA foraging on hot dog baits occurred. Again, despite the similarities in foraging intensity among hay pastures, cattle pastures, and unmanaged habitats, phorid abundance was found to be partitioned based upon habitat.

These aspects of RIFA / phorid associations and habitat partitioning of each species suggest that phorid population densities are not solely dependent upon RIFA population densities. Rather, these data suggest that maintenance of successful phorid populations is possible once a host (RIFA foragers) availability density threshold is met. While this study did not seek to determine this threshold, it appears that the foraging intensity in each habitat except forest exists above it. Evidence of this is provided by the fact that both phorid species were ubiquitous among all but the forest habitat. Further evidence of this is provided by the fact *P. curvatus* appears to be selectively partitioning their population among at least one habitat-specific niche (unmanaged habitat), despite the fact that mean RIFA mound counts were significantly lower in this habitat type than in hay and cattle pastures.

Unquestionably, the difference in degree of habitat heterogeneity among the habitat types surveyed in these trials contributes to differences in a variety of abiotic micro-habitat metrics among these systems, such as relative humidity, degree of shade, and temperature. Additionally, these habitats are likely to support very different communities of invertebrate and vertebrate predators of phorids. An attempt to fully understand the interactions of phorids and abiotic/biotic aspects of these habitats which affect the population dynamics of the flies was outside of the scope of this project. However, the fact that *P. tricuspis* and *P.curvatus* populations are both supported in all but forest habitat indicates that at the time of this study, phorids were capable of co-existence in this system. Additionally, the significantly greater population density of *P. curvatus* observed in unmanaged habitat relative to that of *P. tricuspis* suggests that these habitats may be important for release, long-term success, and expansion of field-released populations of *P. curvatus*. Further, the habitat-specific data related to population densities of *P. tricuspis* suggests that this phorid species may be more compatible with, and more successful in, a wider variety of release site habitats. Further monitoring of this system will provide insight into these unanswered questions regarding long-term sympatric phorid competitive success, and will allow for more accurate strategic planning as it pertains to release and establishment of additional species of *Pseudacteon* phorid parasitoids in the U.S. and elsewhere.

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