Annual Progress Report: Introducing and evaluating species complexes of *Pseudacteon* Phorids for Biocontrol of Imported Fire Ants

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Sub-Project A: Evaluating Impacts of *Pseudacteon* Species Complexes

Major accomplishments achieved to date (September 1, 2005 through August 31, 2006)
• Novel and extremely effective phorid trap developed that allows for measurement of phorid population densities previously below threshold for detection.
• Extensive phorid trapping and monitoring completed yielding detailed population spread and expansion maps. Results from these efforts necessitated a change to the proposed experimental design for assessing the impacts of phorids on *S. invicta* populations (discussed below).

Goals achieved to date (September 1, 2005 through August 31, 2006)
• Plots established and permanently marked at 9 sites in the Gulf Prairie and Balcones Canyonlands regions.
• Fall 2005 sampling completed at 2 sites in Balcones Canyonlands and 3 sites in Gulf Prairie region.
• Spring 2006 sampling completed at all 9 sites. Fall 2006 sampling underway.
• Fall 2005 releases of *P. curvatus* at 2 sites have led to currently established and spreading populations.
• Fall 2006 releases of *P. obtusus* and *P. curvatus* underway at 1 site in Gulf Prairie ecoregion(Brazos Bend State Park).
• First sample on study examining population interactions of *Pseudacteon curvatus* and *Pseudacteon tricuspis* complete. Follow up sampling underway.
• Study on possible mechanisms underlying negative population interactions between *Pseudacteon curvatus* and *Pseudacteon tricuspis* underway.
• Study of variation in host cues used by *P. tricuspis* and *P. curvatus* underway.

Relevance to the Texas Imported Fire Ant Research and Management Project:
The central goal of the studies described in Subproject A is to provide a quantitative assessment of the degree to which these *Pseudacteon* parasitoids impact fire ant populations as well as to document any concomitant impacts on the native ant assemblages that compete with fire ants. Getting these data at this point is particularly critical given the mature state of this biological control effort. Once these phorids have spread across Texas, an accurate assessment of their population level impacts on fire ants may be impossible. Since the past support of the Texas Imported Fire Ant Research and Management Project has been critical to the successful establishment of these parasitoids, understanding their impacts is highly relevant.

Sub-Project B: Culturing, release, and monitoring of *Pseudacteon* species complexees

Major accomplishments achieved to date (September 1, 2005 through August 31, 2006)
• We successfully integrated three species (*P. tricuspis, P. obtusus, and P. curvatus*) into new

mass rearing systems decreasing labor and increasing the total numbers of flies available for release.
• Developed procedures for infecting ants for release experiments and for lab stocks using outdoor *P. curvatus* population at BFL.
• Cultivated and completed host specificity trials and complimentary trials on *P.cultellatus* and *P.obtusus*. Collaborated with Dr Sanford Porter's lab to help identify issues holding *P.cultellatus and P. nocens* from mass production, and obtained first clearance to release *P.obtusus*..
•Discovered pupal diapause in *P. curvatus*.

Goals achieved to date (September 1, 2005 through August 31, 2006)
• Implementation of mass attack rearing system for *P. tricuspis, P. obtusus, and P. curvatus*.
• *P. curvatus* has been released at 10 sites in addition to those described in Subproject A. At this point, it is too early to assess our success at establishing populations at these sites.
• *P. tricuspis* has been released at a total of 8 sites. As this species is now well established through most of central Texas and spreading rapidly (Fig 1), most of these release sites (6) are in South Texas where the challenging abiotic environment has made establishment very difficult. We are pursuing a strategy of releasing in riparian corridors with the intention of establishing populations that may spread out from these relatively mesic environments. In addition, using leveraged funds, an irrigation system has been installed at one of the release sites in South Texas.
• We continued to make progress in cultivating *P. nocens, P. litoralis, P. curvatus, P. obtusus* and the *P. tricuspis* biotype from the arid Santiago del Estero region of Argentina.

Relevance to the Texas Imported Fire Ant Research and Management Project:
Introduction and experimental evaluation of potentially valuable biological control organisms specific to imported fire ant are critical functions allowed by the phorid laboratory at BFL. In addition it provides the capacity to perfect rearing methods and to provide substantial numbers of flies for specificity testing, life history studies, dispersal experiments, genetic study and field release. This is a facility largely created and maintained by the Texas Imported Fire Ant Research and Management Plan.

Publications submitted / published; presentations/posters presented at national technical meetings/conferences:
Presented the results of the study as of last Fall at the 2005 Entomological Society of America Southwestern Branch Meetings, Austin, Texas.

Estrada, C., R. Patrock, P. Folgarait and L.E. Gilbert. 200X. Host Specificity of Four *Pseudacteon* spp. (Diptera: Phoridae), Parasitoids of Fire Ants in Argentina (Hymenoptera: Formicidae). Florida Entomologist (accepted)

Folgarait, P.J. R.J.W. Patrock and L.E. Gilbert. 2006. Development of *Pseudacteon nocens* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* Fire Ants. J.Econ. Entomol. (in press)

Folgarait, P.J., R.J.W. Patrock, and L.E. Gilbert. 200X. The influence of ambient conditions and space on the phenological patterns of a *Solenopsis* phorid guild in an arid environment. Biocontrol (in review)

Kronforst, M.R. P.J. Folgarait, R.J. Patrock, and L.E. Gilbert 2006. Genetic differentiation between body size biotypes of the parasitoid fly *Pseudacteon obtusus* (Diptera: Phoridae) (In Press)

Porter, S.D. and L.E. Gilbert, 2005. Parasitoid Case History: An Evaluation of Methods Used to Assess Host Ranges of Fire Ant Decapitating Flies *In* "Proceedings, 2nd International Symposium of Biological Control of Arthropods" 12-16 September 2005, Davos, Switzerland (M. Hoddle, Ed.)

Signature

Date:
September 8, 2006

Sub-Project A: Evaluating Impacts of *Pseudacteon* Species Complexes : Detailed progress report and explanation of necessary methodological changes

Several changes to the methods and experimental design outlined in the proposal have been required in order to address developments in our understanding of the established phorid populations and to incorporate advances in our abilities to sample these populations. What follows is a detailed description of these changes.

Testing for population level impacts of phorid parasitoids on S. invicta

Originally we proposed testing hypotheses related to how strongly phorids impact *S. invicta* populations by establishing 7 sets of experimental sites: tricuspis only, tricuspis + curvatus, and controls. The two treatment sites in each set were to be spaced so that the tricuspis + curvatus site would be 20 km in front of the advancing tricuspis population. Controls were to be 100 km from release sites. All sites in a set were to be matched for rainfall and vegetation type. However, subsequent intensive phorid trapping and monitoring efforts have revealed that *P. tricuspis* has spread much farther, much faster than we had anticipated at the time of writing the original proposal. Figure 1 provides a conservative estimate of the current distribution of *P. tricupis* in central Texas, and the Gulf region. Prior to these samples, our last reliable estimate of spread was made in 2003. Extrapolating from that, since 2003, *P. tricuspis* populations have been spreading approximately 30 km a year to the north and west of Austin. This rate of spread makes the design presented in the proposal untenable. Sites established 20 km in front of the *P. tricuspis* boundary would be rapidly overrun. Spacing sites with different *Pseudacteon* species compositions far enough apart to allow them to be followed for 4 years, the amount of time that we would like to be able to follow this experiment, would require 120 km spacing. Given the high rainfall gradient in Texas, it is not possible to have sites of comparable biotic and abiotic conditions spaced so far apart.

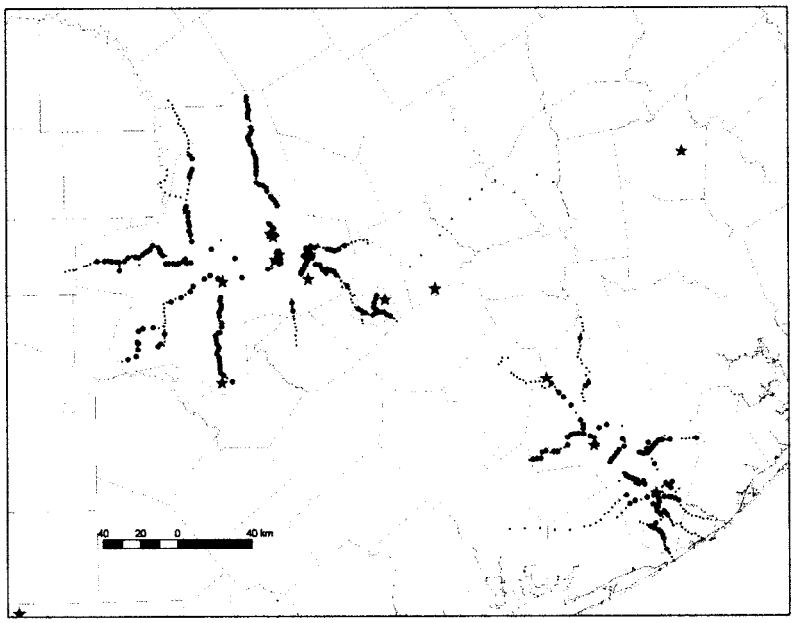

Figure 1: Current extent of *P. tricuspis* populations in Central Texas. Blue polygon is distribution of *P. tricuspis* inferred from the red dots, locations where traps recovered *P. tricuspis*. Stars represent current and past release sites.

Modified Experimental Design

To address these difficulties, we have gone to an experimental design utilizing a more limited number of experimental sites, but these sites are being sampled more intensively. Sites are located within

two biotic provinces: the Gulf coastal prairie and the Balcones Canyonlands. Within each province we have established two treatment sites and two to three unmanipulated control sites. We will employ a repeated measures design to look for an effect of phorids on *S. invicta* population densities and native ant abundance by comparing baseline measurements with spring and fall annual censuses. The significance of any overall effect of phorids on *S. invicta* populations will be evaluated by comparing how consistently different treatment areas respond. All sites have been established and baseline data collected. The long-term control sites will allow us to rule out the possibility that any changes observed in the treatment sites are a result of changes at the landscape scale. In addition, once these sties are eventually overrun by the expanding phorid fly populations, we will be able to use the long-term pre data set to test for any subsequent population changes induced by the phorids.

Established Sites and Progress in Establishing Phorid Populations

Table 1 provides a summary of the release and control sites, and what releases have been accomplished to date and which are planned for 06.

Table 1: Study sites and release schedule

Biotic Province	Site	County	Treatment Category	Releases Fall '05	Releases Spring-Summer '06	Releases Fall '06 (planned)	Currently Established Populations
Coastal Prairie	EC Ranch	Brazoria	*tricuspis+curvatus*	*P. curvatus*	none	Complete	*P. tricuspis* *P. curvatus*
Coastal Prairie	Brazos Bend State Park	Fort Bend	*tricuspis+curvatus+ obtusus*	None	*P. curvatus* *P. obtusus*	*P. curvatus* *P. obtusus*	None (*P. tricuspis* within 1 mile)
Coastal Prairie	Attwater Prairie Chicken	Colorado	control	NA	NA	NA	NA
Coastal Prairie	Armand Bayou Preserve	Harris	control	NA	NA	NA	NA
Coastal Prairie	Peach Point WMA	Brazoria	control	NA	NA	NA	NA
Balcones Canyonlands	Horse Thief Hollow	Travis	*tricuspis+curvatus*	*P. curvatus*	Complete	Complete	*P. tricuspis* *P. curvatus*
Balcones Canyonlands	Pedernales River State Park	Blanco	*tricuspis+curvatus+ obtusus*	None	None	*P. curvatus* *P. obtusus*	*P. tricuspis*
Balcones Canyonlands	Mason Mountain WMA	Mason	control	NA	NA	NA	NA
Balcones Canyonlands	Colorado Bend State Park	Lampasas	control	NA	NA	NA	NA

Modified Methods / Plans for 06:

In the new design all analyses will be performed within sites. In order to have the statistical power to detect changes in this design, we have increased our within site sampling effort. At each site we established two 75 x 45 m plots separated by at least 500 m. Within each

plot we perform 24 bait dominance trials, 24 ecological dominance assays, 24 bait discovery assays, and 24 pitfall traps. The methods for these are the same as in the proposal. Outside of each plot, we take 20 saturation bait samples. The methods for these are the same as in the proposal.

We have developed a fly paper based, passive sampling phorid trap that greatly facilitates the measurement of phorid population densities. These traps are placed into disturbed S. invicta nests, shaded, and left in place for 24 hours. The flies stuck to the paper at the end of this interval can be counted and identified to species and sex. These traps capture approximately an order of magnitude more flies than traditional mound disturbance or tray based monitoring and allow us to measure the densities of phorids in populations that were previously so sparse as to be below the minimum detection threshold (Fig 2). These traps also provide a more standardized sample and eliminate the observer bias associated with traditional mound disturbance sampling. To assess densities of phorid populations in the experimental sites, and to ensure that control sites have not been colonized by phorids, we are deploying 10 phorid traps for 24 hours. This is done in lieu of mound disturbance or tray based sampling.

Do newly introduced *P. curvatus* populations impact established *P. tricuspis* populations?

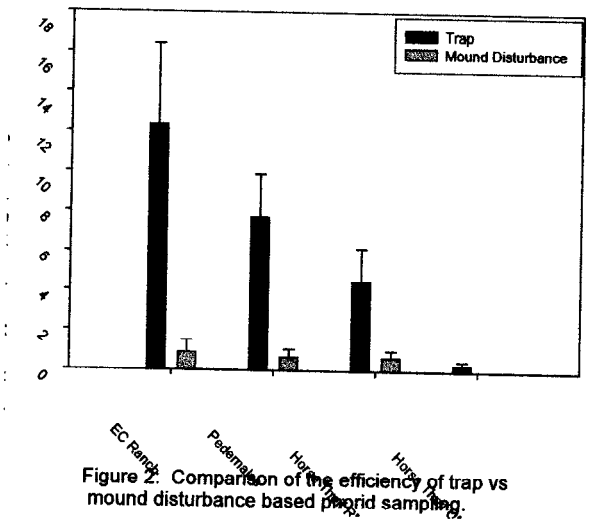

Figure 2. Comparison of the efficiency of trap vs mound disturbance based phorid sampling.

Modifications of Methods and Progress

We have gone to a trap based approach to answering this question. We have identified 11 sites inside the spread boundary of *P. curvatus* within Austin. For each sample we will deploy 10 mound disturbace traps for 24 hours. These data will provide an analysis of any changes in the relative abundance of *P. tricuspis* as sites are colonized by *P. curvatus*. It is too early to be able to separate seasonal effects from real impacts, however, the early patterns in the data suggest that *P. curvatus* may be negatively impacting *P. tricuspis* as it moves into areas previously occupied by *P. tricuspis*. In a second study designed to elucidate the mechanism underlying any population interaction, we are filming mound disturbances at which we have manipulated the species composition of phorids attacking at nest disturbances and are in the process of analyzing these data.

Progress:

We have data on relative abundances of phorid populations for all experimental sites for the spring of 2006 and selected sites for both the spring of '06 and the fall of '05. We will continue this sample this Fall and next Spring and Fall.

Do *P. tricuspis* and *P. curvatus* differ in the contexts in which they exploit *S. invicta* workers?

Progress:

Again we have gone to using phorid traps to answer this question. We are placing an array of 24 bait traps in an area and following this with an array of mound disturbance phorid

traps. We are also measuring the availability of nest mounds and in the habitat as a potential explanatory variable for observed variation in the degree to which these species exploit baits. We have completed a first sample and will undertake repeated samples every other month. Preliminary data indicate that *P. curvatus* attacks *S. invicta* at baits much more commonly than does *P. tricuspis*. Flies will be collected at alate flights opportunistically.

What is the magnitude of direct mortality imposed by *P. curvatus* and *P. tricuspis* on *S. invicta*?

Proposed Methods

To evaluate the efficacy of using midden piles to assess parasitism rates, we will perform treatment blind tests. An observer will sort midden piles into worker heads believed to have been infected by phorids and workers that were not based on morphology. DNA will be extracted from both sets of heads and *Pseudacteon* specific primers will be used to amplify residual DNA left behind by any developing phorid. These data will allow for both a re-evaluation of the direct mortality induced by phorid parasitism as well as yield an understanding of how these direct effects change as additional species of *Pseudacteon* are added into a system. Molecular work will be supported by leveraged funding already in place.

Progress: In collaboration with Alexander Mikheyev of the Ulrich Mueller Laboratory, we have developed phorid specific molecular markers. These will be used to calibrate the morphological assessment of which worker heads have been emptied by emerging phorid. However, progress on this issue has been stalled by a low availability of midden on the soil surface during the recent drought and technical difficulties in amplifying the phorid DNA out of a background of *S. invicta* DNA.

Sub-Project B: Culturing, release, and monitoring of *Pseudacteon* species complexes: Detailed progress report

In the past state fiscal year (Sept. 1 to August 31, 2006) we have maintained the phorid production facility and cultured the following total fly numbers

	PUPAE	FLIES
P. tricuspis	134910	92838
P. curvatus	37694	22890
P. obtusus	19215	12141

In addition we have converted our older more laborious "modular" system into new mass attack systems. We have successfully established all three of the above species into mass rearing systems. The P. *Tricuspis* and *P. obtusus* boxes were of orirignal design and the *P. curvatus* was implemented from USDA design. These mass rearing systems will allow for greater fly production and expeditious procedures reducing the need for technician involvement in certain aspects of rearing. This will greatly increase the capacity of the laboratory to produce large number of multiple species for directed releases at a more conistent output.

We have received three shipments from Dr Sanford Porter's lab. We have collaborated with Porter on establishing *P. cultellatus* to resolve mass rearing issues and are continuously working to integrate this species in the complex of available phorids. We completed host specificity testing on this particular species as well as complimentary trials. *P. obtusus* was also obtained from Dr Porter's lab and to boost the BFL stock. This species has been integrated into the mass rearing system and we have updated our procedures for raising pupae to reflect the specific needs of this species.

In the past fiscal year, we have received three separate shipments from Dr Patricia Folgarait's laboratory in Argentina. We have further investigated *P. nocens* mating strategies and pupal development to help establish F2 laboratory generations. We also collaborated with Dr Porter to share information to help establish this species in the laboratory. We also directed efforts to bring *P. tricuspis* from Santiago del Estero biotype into rearing. We will continue to work with Dr Folgarait on bringing this biotype to the laboratory. This will require additional stock in the future to help boost total laboratory numbers.

Total for All Shipments	
P. cultellatus	1025
P. curvatus	32
P. litaloris	95
P. nocens	285
P. obtusus	4677
P. tricuspis	417
P. nudicornis	1
P. solenopsidis	1
Males	1302

In addition to meeting the above objectives, the laboratory also successfully developed procedures to utilize the established field population of *P. curvatus* to infect ants from field releases sites and to boost the laboratory stock. This new implementation will help to further the spread of *P. curvatus* with minimal input from laboratory cultures. This infection strategy is also being used to infect ants with *P. obtusus* to allow full utilization of each female's reproductive ability within the confines of the optimal laboratory conditions.

The laboratory has experimented throughout the year to further optimize the pupal condition of all species and lineages to increase our pupal to adult emergence rate. During the investigation, we discovered a pupal diapause in *P. curvatus* and the laboratory will continue to experiment as to the cause and condition of the diapause and the field impacts of this behavior.