

Proposal FY 2003-2005 Competitive Grants: Biological Control

Title: Research on Applying Phorid Flies to the Biological Control of Red Imported Fire Ant, *Solenopsis invicta*, in Texas

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Introduction

Our immediate goal is to fully evaluate the potential of *Pseudacteon* phorid flies as agents for biological control of imported fire ants in Texas. Previous work showed the feasibility of importing and laboratory rearing of exotic South American species of *Pseudacteon*. However, the first strain released widely in Texas, South Brazilian *P. tricuspis*, although naturalized in places, did not initially thrive and spread as it did in Florida. This species has recently expanded dramatically in abundance and distribution in sites where regular rainfall interrupted drought but not in sites at which dry conditions prevailed between Fall '02 and Spring '03. We will continue tracking the patterns of success and failure at *P. tricuspis* release sites with funding from private grant sources. The work proposed here is focused on discovery, evaluation, and lab production of new *Pseudacteon* species or ecologically distinct varieties of *P. tricuspis* which show promise of thriving and impacting *Solenopsis invicta* in Texas. Under **Sub-Project A**, we will continue with collaborators, to prospect for, and rear in Argentina for export, new species and biotypes of *Pseudacteon*. Under **Sub-Project B** our laboratory's main focus will be on introducing to Texas these additional species and strains of *Pseudacteon* whose geographical origin or life histories might pre-adapt them for success in ecological zones of Texas and/or the polygynous colonies of *S. invicta* that characterize Texas populations of imported fire ant.

Sub-Project A: Prospecting in South America for additional *Pseudacteon*

phorid species for export to and release in the U.S. against imported fire ants.

Background

There are approximately 20 species of *Pseudacteon* phorids known to attack *S. invicta* and its close relatives in South America. These occur locally as suites or "complexes" of five or six phorid species. Furthermore it is common to observe two or three phorid species simultaneously attacking worker fire ants around baits or disturbed mounds. Because the species of a local phorid community differ in sizes of workers attacked and in host finding tactics, it has long been suggested that the full potential of *Pseudacteon* as a factor in the biological control of fire ants will only be achieved by employing several complementary species together (Orr et al 1997).

In 1999, we concluded an NSF grant that had funded much of our fieldwork in Brazil and had provided cooperators who helped us export phorids from Brazil to the Texas. Unfortunately, changes in Brazil's policies towards research and collecting subsequently made work there more difficult. Although there are still potential species of interest in southern Brazil, and policies have loosened somewhat, the difficulty of establishing *Pseudacteon* from tropical Brazil in Texas during recent drought years let us to focus more on habitats at appropriate latitudes in Argentina. Meanwhile, a former postdoctoral researcher on our project has become established in Argentina as a faculty member at the National University of Quilmes, near Buenos Aires.

Over the last several years and with private foundation grants and the Texas Imported Fire Ant Research and Management project, we have assisted Folgarait establish the first South American Laboratory devoted to the rearing and study of *Pseudacteon* phorid flies. The results of that effort can be seen in a series of recently published or "in press" papers (all Folgarait et al.) on the life history, host range and seasonal activity of Argentine *Pseudacteon* species. Folgarait and I were able to collect and culture several new species as well as eco-types of *P. tricuspis* from harsh localities in north-central Argentina, selected *a priori* based on their Texas-like climates. Some of these phorids have been introduced to our quarantine facility at BFL and we have maintained one of these since May 2001.

The Argentina project was slowed somewhat by the recent economic crisis in Argentina. However, leveraging of Texas funds by Argentinean sources has been remarkable. First, Folgarait's position in her university was made secure during a time of faculty lay-offs, second, Folgarait was given additional space to remodel for a phorid rearing room, third, The University of Quilmes provided her with a new field vehicle for collecting and research trips, and fourth, two of her graduate students won CONICET fellowships for Ph.D. studies. One of these was a technician in the phorid lab and now will do research in the phorid system. In addition to establishing field sites for collecting in the States of Santiago del Estero and Corrientes, the project has trained local people to collect and ship live phorids and ants to the University of Quilmes near Buenos Aires.

In the last two years we accomplished many things including discovery and description of an undescribed *Pseudacteon* (*P. bulbosus*), and the characterization of several *Pseudacteon* species that hold promise as candidates to test against imported fire ants in Texas. We also have

evidence of "cryptic species" i.e. morphologically identical taxa which are sexually isolated as distinct species. For various reasons beyond our control, mass rearing of phorids at Quilmes was not accomplished until recent completion of the environmental controls in the Folgarait laboratory. Consequently, our goal of establishing certain rare trail-attacking species in our Austin lab by first establishing source cultures in Argentina has not yet been fulfilled (although we still have five months remaining in the current project).

Objectives and Methods

The objectives of this long term project are to discover, collect, study and mass rear Argentinean species of the phorid genus *Pseudacteon* whose habitats and habits qualify them for exportation to and specificity testing in our laboratory in Texas or at S. Porter's facility in Gainesville. The ultimate goal of this project is to produce phorid pupae in sufficient number to allow establishment of a variety of species in culture in Texas and elsewhere. Of these species, candidates deemed most likely to complement *Pseudacteon* already naturalized in parts of the U.S. will be developed as laboratory cultures for release under separately funded projects in Austin and/or Gainesville.

Both harsh and uncertain climate and the multiple-queen (polygyne) nature of its fire ant populations may be constraints to the introduction to many parts of Texas of larger tropical phorids like Brazilian *P. tricuspis* (Gilbert and Patrock, 2002). Therefore in this project we focus our attention on several life history attributes of prospect phorid species that may increase the likelihood that they will thrive on fire ants in Texas.

Imported fire ants, but not native fire ants, remain active through Texas winters (Wuellner and Saunders 2003). We plan to investigate further the attributes of *P. borgmeiri*, an Argentinean species that remains active in cool weather, as well as other species adapted to dry conditions. We will bring into culture as soon as possible several trail-orienting phorids for later testing and possible release trials. We will continue to target polygyne populations of *S. invicta* in Argentina to find out which phorids (if any) do well breeding on polygyne fire ants.

The same methods of rearing phorid flies used in the Austin phorid facility have been duplicated and then modified slightly in the Argentina laboratory. We use standard field collecting techniques in which individual flies are aspirated into small individual tubes for identification, storage and transport.

References

- Brown, B. V., P. Folgarait, L. Gilbert, A new species of *Pseudacteon* Coquillett (Diptera: Phoridae) attacking *Solenopsis* fire ants (Hymenoptera: Formicidae) in Argentina (In review).
- Folgarait, P.J., Bruzzone, O.A., Patrock, R., Gilbert, L.E.. 2002a. Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. *J. Econ. Entomol.*, **95**: 1151-1158.
- Folgarait, P.J., Bruzzone, O.A., Gilbert, L.E. 2002b. Development of *Pseudacteon cultellatus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants. *Environ. Entomol.*, **31**, 403-410.

- Folgarait, P.J., Bruzzone, O.A. Pesquero, M.A., Gilbert, L.E., Porter, S.D. 2000. Geographic ranges and patterns of diversity for *Pseudacteon* flies, parasitoids of *Solenopsis* ants from Argentina and Brazil. *XXI International Congress of Entomology*, 1, 126.
- Folgarait, P. J.,* Bruzzone, O. A.,* and Gilbert, L. E. (XXXX) Seasonal Patterns of Activity Among Species of Black Fire Ant Parasitoid Flies (*Pseudacteon*: Phoridae) in Argentina Explained by Analysis of Climatic Variables. (In review)
- Gilbert, L.E., Patrock, R.J.W. 2002. Phorid flies for the biological suppression of imported fire ants in Texas: Region specific challenges, Recent Advances and Future Prospects. *Southwest. Entomol.*, 27, 7-17.
- Orr, M.R., S.H. Seike, and L.E. Gilbert. 1997. Foraging ecology and patterns of diversification in dipteran parasitoids of fire ants in south Brazil. *Ecol. Entomol.* 22: 305-314.
- Wuellner, C.T., Saunders, J. 2003. Circadian and Circannual Patterns of Activity and Territory Shifts: Comparing a Native Ant (*Solenopsis geminata*; Hymenoptera: Formicidae) with Its Exotic, Invasive Congener (*S. invicta*) and Its Parasitoids (*Pseudacteon* spp., Diptera: Phoridae) at a Central Texas Site. *Ann. Entomol. Soc. Amer.*, 96, 54-60.

Time Line

Fall 2003 to Early Winter 2004:

- Introduce two new species to the BFL facility from stocks already produced in the Folgarait Lab or easily collected. Porter and Gilbert group will make collecting trips.

Fall to Spring 2004:

- Field work with Folgarait team and additional exploration for field sites
- Continue introducing two additional species in Austin as numbers in the Ouilmes lab cultures allow.
- Gilbert and Porter will make a join trip in mid Fall

Summer 2004 & Summer 2005:

- Summer in Texas is winter in Argentina. These are periods for work on specificity testing the laboratory cultures and tailoring culture methods to new species.

Expected Outcome

This project aims to discover and test several additional species or biotypes of *Pseudacteon* from Argentina per year for possible biological control trials in Texas and elsewhere. Within two years we expect to have added at least two species of *Pseudacteon* (in addition to biotypes or sibling species of *P. tricuspis* from Texas-like regions of Argentina) in selected areas where Brazilian *P. tricuspis* have previously been released.

Relevance to the Texas Imported Fire Ant Research and Management Plan

A key to evaluating the potential of phorid flies in the biological control of phorid flies is testing the best possible species or combinations of species for each ecological region where imported fire ants are serious pests. We are focused on parts of Texas where *P. tricuspis* has proven difficult to establish or slow to spread once established. Only with access to the diversity of

Pseudacteon species and biotypes provided in climatic zones of Argentina that parallel those of Texas and the SE U.S. will we be able to know if we have given phorid flies a fair opportunity to prove themselves capable of exerting population-level controls on fire ants.

Collaborations - Related Projects, Funding and Support

Because of the unique needs of funding a collaborating laboratory and field operation in Argentina, we employ a mechanism suggested by the UT business office and approved by auditors that allows Gilbert to guide the work of the Argentina phorid lab and field collecting program in consultation with Folgarait who, in return participated under a formal contract s from several sources including private foundation grants and the Texas Imported Fire Ant Research and Management Project. It is critical to the goals of the Texas fire ant project to keep this struggling (*but highly productive*) program going in Argentina by incorporating several sources for its support. This approach buffers against the "on-off" nature of short term funding.

Private foundations whose funds have played an important role in establishing and maintaining the Folgarait collaboration include the Helen C. Kleberg and Robert J. Kleberg Foundation, the Fondren Foundation, the Houston Livestock Show and Rodeo (HLSR). HLSR has ceased funding this research, at least temporarily. The Fondren grant has expired and the Kleberg and Kleberg funds expire in June '03. The amount budgeted above is the current support provided Folgarait under the UT contract plus funds to support travel. I will be applying to renew foundation support of part of this project. Any support above baseline will allow additional exploration. The relevant area of Argentina is vast and this program only scratched the surface.

Sanford Porter is currently collecting in other sites in Argentina. We stay in contact and are prepared to join forces to maintain important stocks of *Pseudacteon* uncovered in these field surveys. Occasional overlap on field trips will function to improve communication and coordination on deciding which species or biotype will be reared where. Note (above) that we have already joined with Porter in summarizing biogeographical data on South American *Pseudacteon*.. We budget for support of one trip per year for Sanford Porter so that he can consult with Folgarait and collaborate with LEG in field collections.

Budget Justification

Over the last few years we have established that the field and laboratory work needed to screen for potential new *Pseudacteon* for biological control of fire ants in Texas is most efficiently and cost-effectively accomplished done by collaborators in Argentina. The current agreement that allows funds to be transferred for that purpose is appended. We propose to continue that agreement since it specifies research goals which mesh precisely with this proposal. The agreement with Folgarait supports her phorid laboratory , including salaries of lab technicians , research on phorid biology relevant to project goals and supports field exploration, collection of ants and phorids for the research (field collectors in three states, B.A., Corrientes and Santiago del Estero). It covers local purchase of supplies, in country field travel, per diem, maintenance of vehicles and environmental chambers.

Since this proposal was submitted, private funding was received which can cover the cost of the original agreement. Additionally, since the initial proposal was submitted, serious health issues have emerged which will reduce Folgarait's capacity to conduct field work. Thus in this budget I am allocating a reduced amount, \$15,000, as a contribution to the cost of the agreement and \$30,000 for a postdoctoral associate to assist in the Argentina field and laboratory work.

Foreign Travel: Field trips by Gilbert's group and Porter to Argentina and Folgarait's group to Austin are absolutely critical for helping guide the work and for hand carrying valuable live stocks of phorids to Texas from Argentina. This is particularly critical now that shipments of materials are exposed to strong x-rays. Four round trips between B.A. and the U.S. @ \$2K per trip are included for the team per year. Additional funds will be required for extended field trips and private grants already obtained will cover these additional expenses.

Sub-Project B: Laboratory Production, Strain Improvement and Specificity Testing of South American *Pseudacteon*

Background

Our phorid laboratory at BFL, funded from a variety of sources, continues to culture several strains of *Pseudacteon tricuspis* for release in Texas and for use in experimental work on basic biology of *Pseudacteon* (e.g. Morrison, Porter and Gilbert, 1998 and Consoli, Wuellner, Vinson and Gilbert, 2001). Between May 2001 and present, we have maintained a stock from Santiago del Estero, Argentina. This strain is of great interest because it lives in habitat very similar to South Texas in climate and vegetative physiognomy, and may have an egg or early larval diapause in the ant host which allows the population to survive harsh periods without adults present (much as native Texas *Pseudacteon* do between December and April). Based on our finding that time from egg to pupa in this biotype can be three months (versus just over one month in the Brazilian stocks) In spring of 2003 we adopted a *P. tricuspis* stock (Formosa, Argentina) from Porter's lab which we plan to expand and test in Texas.

Over the years we have been able to breed *P. curvatus* and *P. cultellatus*, two very minute species and we've kept nine or more species of the genus in the facility long enough to conduct specificity trials (e.g. Gilbert and Morrison 1997) and studies of differential ant responses to the attack by females of different species (Wuellner et al 2002). However, for many interesting species, such as trail orienting phorids like *nudicornis* and small *obtusus*, we have been disappointed not to be able to collect and import the necessary critical numbers needed to initiate cultures. Our lab has now adopted the strategy of conducting much of the basic life history work in collaboration with the Folgarait lab in Argentina (**Sub Project A**) and we will grow up sufficient numbers in her laboratory to achieve the necessary starting population.

For the near term we will stick with a labor-intensive method of rearing phorids because it allows us to keep data on experimental groups. Thus, for example, we can modify the ratio of large to small ants presented to a set of females and check the impact on fly sex ratio. Or, we can

see whether selection for small females leads to a correlated preference by those females for smaller ants than is the case for unselected strains. Our method of rearing also allows us to "trouble shoot" to find out why a particular stock is not doing well. Best of all it will allow us to make a gradual transition from emphasis on one mass-produced stock to another. We will phase out most strains of *P. tricuspis* gradually as they establish source populations outdoors and shift to strains of "*P. tricuspis*" from more Texas-like area of Argentina as well as any additional host specific species for which release permits are obtained.

Sanford Porter and I have and will continue to coordinate efforts to increase the chances that important but difficult species get established in one lab or the other and we will maintain back-up stocks to reduce the chance of extinction (that will be cheaper than going to South America for another collection.) Our labs also will cooperate in speeding up the evaluation of potential candidates for release. Nevertheless, the laboratories agree to retain a degree of independence in terms of which species to emphasize.

Objectives and Methods

Our objectives are as follows:

- 1) to maintain a phorid production facility, which continues to provide for field release experiments as well as experimental work needed to develop novel rearing methods, or to evaluate the specificity of possible candidates for release.
- 2) to collaborate with Porter's lab towards the goal of adding up to four *Pseudacteon* species to the list of those permitted for release in the U.S. during '03-'05.
- 3) to replace Brazilian *P. tricuspis* with Argentinean biotypes/sister taxa of that species in previously unsuccessful release sites,
- 4) to complement established *P. tricuspis* sites with one or more small species, including a trail orienting species.
- 5) to prioritize for production at BFL phorid species whose life history characteristics are most likely to overcome constraints though to prevent the successful spread of Brazilian *P. tricuspis*.
- 6) to cooperate with appropriate phorid labs in Florida and Mississippi to make sure that species brought by us from Argentina are directed to the region where they will be most appropriate.

For *Pseudacteon* species that mate above ants in attack trays, the culture methods will be similar to those established for *P. tricuspis*. For species which utilize other situations for mating we will test a variety of possibilities using novel arena designs not yet developed. Such work is currently in progress with *P. nocens*. Once mated, *P. nocens* females attack normally. Specificity trials will follow the "sequential no choice" method we have previously utilized. (Gilbert and Morrison 1997). Potential new stocks will be brought to Austin either as pupae reared in the Folgarait laboratory, as or as adults in

References

Cônsoli, F. L., C. T. Wuellner, S. B. Vinson, and L. E. Gilbert. 2001. Immature development of *Pseudacteon tricuspis* (Diptera: Phoridae), an endoparasitoid of the red imported fire ant (Hymenoptera:

- Formicidae). *Ann. Entomol. Soc. Am.* 94: 97-109.
- Gilbert, L.E. and L.W. Morrison. 1997. Patterns of host specificity in *Pseudacteon* parasitoid flies (Diptera: Phoridae) that attack *Solenopsis* fire ants (Hymenoptera: Formicidae). *Environ. Entomol.* 26: 1149-1154.
- Folgarait, P.J., Bruzzone, O.A., Gilbert, L.E. 2002. Development of *Pseudacteon cultellatus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants. *Environ. Entomol.*, 31, 403-410.
- Morrison, L.W., S.D. Porter, and L.E. Gilbert. 1999a. Sex ratio variation as a function of host size in *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). *Biol. J. Linn. Soc.* 66: 257-267.
- Wuellner, C., C. G. Holvorcem, W.W. Benson, and L.E. Gilbert. 2002. Phorid fly (*Pseudacteon* spp.) oviposition behavior and attacked red imported fire ant response differ according to fly species. *Ann. Entomol. Soc. Amer.* 95: 257-266.

Time Line

The phorid laboratory by necessity must function 7 days a week, 365 days a year. Unless we discontinue the operation, 90% of the activity going on this week will be going on for the next two years. About all I can predict it that the opportunity for bringing up different species is best from November to May when Argentina has spring and summer.

Spring - Summer 2003:

- Prior to the starting date we hope to introduce *P. cultellatus*, and *P. obtusus* from the Folgarait laboratory. (August '03 update: we have imported *P. nocens* and a population of *P. "obtusus"* which AFLP analysis (using wingless gene) shows to be a sister species of the small *obtusus* of Brazil and N Argentina.

Fall 2003:

- Conduct specificity tests on *P. nocens*, "large" *P. obtusus*, *P. cultellatus* (and an additional *P. obtusus* "complex" stock from Porter laboratory?). Conduct specificity tests with *tricuspis* stock "2-4" from Santiago del Estero, Arg. The latter may be an un-described sister species of *tricuspis*.

Spring 2004:

Transfer extra *P. cultellatus* to Porter's laboratory to establish a culture there. Add additional stocks of *P. tricuspis* from Santiago del Estero Argentina and "small *obtusus*" since it now proves to be a different species.

Summer 2004:

- Import Argentina's "winter phorid" *P. borgmeieri*.

Fall 2004-Spring 2005:

- Small "obtusus", a host specific trail phorid, already past will be the next priority for lab production. Produce *P. cultellatus* in numbers adequate for field release.

Summer 2005:

- Several additional *Pseudacteon* species and/or biotypes naturalized in Texas.

- 1) **To begin release of new species and biotypes and they become available from the Argentina work and the phorid rearing lab project. To monitor which species and strains are successful using AFLP techniques developed on last project. Methods:** Although female *Pseudacteon* of different species can be distinguished, males are almost identical and can only be identified by AFLP. Also if different strains of *tricuspis* are released in an area we will use this technique to sort them out. A graduate student, Marcus Kronforst, trained on a previous project will conduct the assays if chemicals are purchased.

References

Gilbert, L.E. and R.J.W. Patrock. 2002. Phorid flies for the biological suppression of imported fire ants in Texas: Region specific challenges, recent advances and future prospects. *Southwestern Entomologist*. Suppl. No. 25:7-17.

Time Line

Spring - Summer 2003:

- Continue ongoing releases and monitoring activity. Continue to measure ant worker size distribution across all sites and assemble weather records.

Fall 2003:

- Analysis of all release data (Texas plus USDA)

Spring 2004:

- We anticipate being able to initiate releases of additional *Pseudacteon* species in a few sites.

Summer 2004

- Continued monitoring

Fall 2004–Spring 2005

- We hope to be fully in transition to releases of several species other than *P. tricuspis*

Summer 2005:

- write up work

Expected Outcome

We expect to bring four or more additional species or biotypes of *Pseudacteon* phorids through specificity tests and into laboratory production.

Additional *Pseudacteon* species which complement *P. tricuspis* will be “layered” into previous release sites of *tricuspis* across the range of fire ants in Texas.

Relevance to the Texas Imported Fire Ant Research and Management Plan

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 field release. This is a facility largely created and maintained by the Texas Imported Fire Ant Research and Management Plan.

Collaborations - Related Projects, Funding and Support

Funding external to those provided through FAARMAC will be sought as appropriate to accomplish overall goals of the project. Since the early draft of this proposal funding from the Robert J. and Helen C. Kleberg Foundation has become available. This support will allow some of the priority projects removed from the original proposal to proceed.

According to the interests of Post Doctoral personnel recruited, applications will likely be made to NSF and USDA during the course of the funding period in order to pursue important ancillary questions stimulated by some of the ongoing work.

Budget Justification:

The 1 1/2 time salary requested for postdoctoral and research associate-level personnel is critical for the coordination, analysis and communication of the experimental work across all sub-projects. Such personnel are especially important because P.I. Gilbert's time to concentrate on the project is fragmented (L.G. teaches at UT Austin, directs six unrelated Ph.D research projects, and directs the Brackenridge Field Laboratory). The technical assistants requested are based on current requirements of the phorid laboratory (three full time techs plus two half time students for the rearing) , (one full time technician for specificity testing), (one and one half full time field assistant for ant collection and care), and general project support Domestic travel for field work and budgets for materials and supplies are projected from past expenditures. Equipment and vehicles acquired early in the first six year phase of the project are beginning to require repair. Thus funds are requested to cover such needs.

	FY 2003	FY 2004	Total
A. Personnel (indicate number of people in parentheses)			
1. (1) Principal Investigator/Principal Co-Investigator	7,500	7,500	15,000
2.(1.5) PostDoc/Research Associate	50,000	50,000	100,000
3. (4) Other Professionals (Technicians, Programmers, etc.)	100,000	102,000	202,000
4. (0) Graduate Students (half time)			
5. (1) Undergraduate Student (half time)	9,000	9,000	18,000
6. () Secretarial-Clerical			
7. () Other			
Total Salaries and Wages	166,500	168,500	335,000
C. Permanent Equipment			
Total Permanent Equipment			
D. Travel			
1. Domestic (air, ground, & per diem)	3,000	3,000	6,000
2. Foreign (round trips: 3, LEG ; 1 SP per year)	8,000	8,000	16,000
Total Travel	11000	11000	22000
E. Other Direct Costs			
1. Materials and Supplies	3,500	3,500	7,000
2. Publication costs/ page charges	2,000	2,000	4,000
3. Consultant Services			
4. Computer Services			
5. Maintenance & Repairs of vehicles & equipment	2,000		2,000
6. Other (Agreement with Folgarait/Argentina)	15,000	15,000	30,000
Total Other Direct Cost	22,500	20,500	43,000
Total Direct Costs	200,000	200,000	400,000