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Dear Kevin

Texas Imported Fire Ant Research Ant Management Project

I am pleased to submit our report for the FY2006-2007 Texas Imported Fire Ant Research Ant Management Project.

Sincerely

Robert M Plowes, PhD
Research Associate
Brackenridge Field Laboratory

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

Principal Investigator: Lawrence Gilbert, Brackenridge Field Laboratory, UT Austin

Co - PI: Edward LeBrun, Brackenridge Field Laboratory, UT Austin

Research Coordinator: Robert Plowes, Brackenridge Field Laboratory, UT Austin

<u>**Lay Summary of Major accomplishments (September 1, 2006 through August 31, 2007)**</u>

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

• Completed base line assessments of imported fire ant and native ant densities for all 9 experimental sites in Fall 2006 and Spring 2007. Sampling yielded 4 independent measures fire ant population density as well as detailed information on the prevalence of native ant species and the dynamics of resource competition between the native ant species and *S. invicta*. The relative abundance of phorid species also measured twice annually. Sampling will resume once sufficient time has elapsed for any effect on *S. invicta* densities to be realized.

• Releases of phorid populations successfully completed at the 9 experimental sites yielding 1 site with 3 *Pseudacteon* species (*obtusus, curvatus, tricuspis*), 3 sites with 2 *Pseudacteon* (*curvatus, tricuspis*), 1 site with 1 *Pseudacteon* species (*tricuspis*), and 4 sites that currently lack *Pseudacteon*. This summer efforts will continue to establish *P. obtusus* at a second site (Pedernales Falls SP).

• Extensive phorid trapping completed yielding a detailed understanding of mechanisms by which populations spread and the current extent of introduced *Pseudacteon* populations. These novel perspectives on expansion and interactions that will help chart future studies and programs on this project in Texas.

• Documented and characterized the suppression of *P. tricuspis* populations by expanding *P. curvatus* populations in central Texas. Experiments are in progress to elucidate the causal mechanisms.

• Other recent studies show how environmental factors and competition from native fire ants may affect local densities of *S. invicta* (Plowes et al. 2007).

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

• First population of *P. obtusus* in North America established at Brazos Bend State Park.

• Broad success in establishing *P. curvatus* at nine sites in south Texas using a modified infection method where previous infections with *P. tricuspis* has generally failed.

• Sampling revealed that the spread of well established *P. tricuspis* populations is dominated by the prevailing wind, which generates effective, long-distance dispersal over tens of kilometers leading to population spread rates of 40 - 70 km per year. The first successful releases of *P. tricuspis* now cover most of central Texas.

• First releases of *P. nocens* in North America, still pending confirmation of establishment.

• Continued success in importing regular shipments of phorid flies from Argentina, using a method of direct shipment of field attacked ants.

• We performed the first releases of intact communities of flies collected in Argentina. This is intended to reconstruct the suite of flies found associated in a native system, since we hypothesize that a community of several species of flies will have a broader biocontrol impact on fire ants.

• A permit has been received to release a broad array of *Pseudacteon* species in Texas that have previously been tested for specificity.

• Lab based production of phorids successfully moved to mass attack chambers for improved efficiency.

<u>Relevance to Achieving the Overarching Goals of the Texas Imported Fire Ant Research and Management Project:</u>

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. Once these phorids have spread across Texas, an accurate assessment of their population level impacts on fire ants may be difficult since we will lack experimental control sites, and our strategy is to perform longitudinal temporal studies using a range of baseline surveys established in this study. 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.

Introduction and experimental evaluation of potentially valuable biological control organisms specific to imported fire ant are critical functions performed 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.

Our release efforts have resulted in the establishment of 3 species in Texas, with additional species in process. The first species to be released, *P. tricuspis,* has now spread over 7 million acres in Texas, and the successful establishment of the other species will result in multi-species communities of flies in Texas. These phorid communities are considered essential for effective biocontrol of fire ants since a wider range of niche space is covered. These releases are an integral part of the Texas Imported Fire Ant Research and Management Plan.

We have successfully leveraged support from this program to secure additional funds from private donors. Such funding allows us to cover a wider scope of synergistic activities that benefit the long term objectives of the State program. The scale of our operation includes fly production, release activities, acquisition of additional species in Argentina, lab and field based experiments, all of which are interlinked. Any reduction in funding from either private or State sources will curtail this broad scope of work.

Manuscripts published/in press/submitted

Folgarait, P.J., R.J.W. Patrock and L.E.Gilbert. Submitted. The influence of ambient conditions and space on the phenological patterns of a *Solenopsis* phorid guild in an arid environment. Biological Control.

Folgarait, P.J., R.J.W. Patrock and L.E.Gilbert. Submitted. Associations of fire ant phorids and microhabitats: the influences of light, host location and ambient conditions.

LeBrun, E.G., R.M. Plowes, and L.E. Gilbert. In press. Dynamic expansion in recently introduced populations of fire ant parasitoids (Diptera: Phoridae). Biological Invasions.

LeBrun, E.G., J.S. Sprague, and L.E. Gilbert. In review. An improved, trap-based technique for sampling populations of Phorid fly (Diptera: Phoridae) parasitoids of ants. Insectes Sociaux.

Plowes, R. M., J. G. Dunn, and L. E. Gilbert. 2007. The urban fire ant paradox: native ants persist in an urban refuge while invasive ants dominate natural habitats. Biological Invasions, in press. DOI 10.1007/s10530-006-9084-7.

Gilbert, L. E., C.L. Barr, A.A. Calixto, J.L. Cook, B.M. Drees, E.G. LeBrun, R.J.W. Patrock, R.M. Plowes, S.D. Porter, and R.T. Puckett. Submitted. Introducing phorid fly parasitoids of red imported fire ant workers from South America to Texas: Outcomes vary by region and by *Pseudacteon* species released. S.W. Entomologist.

Invited and Submitted Presentations/Posters Presented at Scientific/Technical Meetings/Conferences:

LeBrun, E.G., R. Plowes, and L.E. Gilbert. 2007. Dynamic spread in recently introduced populations of fire ant parasitoids (Diptera: Phoridae). Southwestern Branch Meeting of the Entomological Society of America. Corpus Christi, TX.

Signatures **Date:**

August 1st, 2007

L E. Gilbert

E. G. LeBrun

R. M. Plowes

Technical Description on Progress on Individual Objectives

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

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

During this funding year (September 1, 2006 through August 31, 2007), we completed the fall 2006 and spring 2007 protocols for measuring *Solenopsis invicta* densities, native ant densities, phorid fly densities, and the foraging dynamics of both native ants and introduced fire ants. These protocols were completed at the 9 sites listed in Table 1, with 2 plots per field site. The protocols are described in detail in the original proposal.

We have also largely completed establishing populations of the planned species assemblages of phorid flies at our treatment sites. During this funding period, we succeeded in establishing *P.* curvatus at two of our study sites and *P. obtusus* at one. The anticipated colonization by expanding *P. tricuspis* of two more sites also occurred. The population of *P. obtusus* established at Brazos Bend State Park represents the first population of this species established in North America. Table 1 outlines all the sites and the progress to date in establishing the desired *Pseudacteon* species composition. The only remaining population that we are still attempting to establish for this study is *P. obtusus* at Pedernales Falls State Park.

Table 1: Study sites and release schedule

Biotic Province	Site	County	Treatment Category	Releases Fall '05	Releases Spring-Summer '06	Releases Fall '06	Releases Spring – Summer '07	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*	*P. curvatus* *P. obtusus*	*P. tricuspis* *P. curvatus* *P. obtusus*
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. curvatus* *P. obtusus*	*P. tricuspis* *P. curvatus*
Balcones Canyonlands	Mason Mountain WMA	Mason	control	NA	NA	NA		NA
Balcones Canyonlands	Colorado Bend State Park	Lampasas	control	NA	NA	NA		*P. tricuspis* (colonized July, 2007)

For the 18 permanent plots at 9 sites we now have 4 measures of *S. invicta* density (pitfall trap samples, saturation baiting samples, prevalence at baits, and mound density measurements), detailed information on the density and distribution of native ants, information on competition between native ants and fire ants, and densities of phorid flies for three time intervals: spring 2006, fall 2007, and spring 2007. We added the fourth measure of fire ant population densities, mound size and density, in the spring of 2007 using methods described by Morrison et al (2005). With these data in place we are well situated to evaluate how fire ant densities respond to the long-term presence of newly established phorid fly populations of varying composition.

Timeframe for detecting impacts

Phorids have immediate behavioral impacts on their hosts, such as reduced foraging. But, they attack the sterile worker caste, and thus do not immediately remove colonies from the environment. Impacts on population densities of their host will require time to be realized. In the presence of vibrant, species rich assemblages of phorid parasitoids, fire ant colonies can be expected to face reduced foraging opportunities and increased worker mortality. Over the growing season, these effects will translate into reduced resources to allocate towards alate production and colony growth. However, several years of this type of impact may be required before an effect is apparent at the level fire ant population density.

To allow for any effects these parasitoids may have on *S. invicta* population densities to be realized, our intention is to allow two years from the establishment of the last *Pseudacteon* species at a site and then take an end point sample to evaluate changes. Our samples have revealed a reasonably high amount of inter-season and inter-annual variation in *S. invicta* densities at a site. To account for this variation, our end point sample will also include a paired comparison with sites near each of the original 9 study sites that are uncolonized or only recently colonized by *Pseudacteon.* In order to assess the times which sites have been occupied, we have begun the tracking of populations spreading out of all study sites and will continue this effort.

<u>Do newly introduced *P. curvatus* populations impact established *P. tricuspis* populations?</u>

Using mound-disturbance phorid traps we collected data on the relative abundance of *P. tricuspis* and *P. curvatus* at 20 sites around the Austin area in the spring and fall of 2006 and again in June-July of 2007. Within 6 months of arrival at a site, *P. curvatus* populations achieve densities greater than 10x local *P. tricuspis* populations. This asymmetry is not simply the result of population increases by *P. curvatus*. *P. curvatus* greatly reduces the abundance of *P. tricuspis* at a site within one-year of its arrival. As you move out from the center of the *P. curvatus* distribution to more recently colonized and therefore less strongly

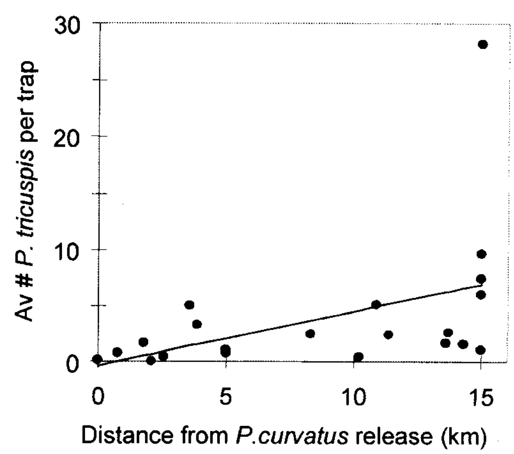

Fig 1: *P. tricuspis* densities decrease following release and spread of *P.curvatus*.

impacted sites, *P. tricuspis* densities increase (Fig 1). Analyses of these data as well as longitudinal comparisons of changes within sites across years are ongoing. Information that will be extracted from the data include: the approximate speed at which this displacement occurs, seasonal variation in the rate of displacement, and the densities *P. tricuspis* is able to maintain one *P. curvatus* reaches maximum abundance.

The mechanism underlying this displacement may be indirect competition via pre-emption of access to appropriately sized hosts, and the intensity of this impact may vary seasonally. We have begun a study looking at this mechanism and at potential direct competition between these species for the smaller sized workers. Preliminary results indicate that following attack by either *P. tricuspis* or *P. curvatus* workers retreat into the nest mound reducing the availability of workers to attack. This is true for all worker size classes regardless of the identity of the attacking fly (Fig 2). However, in central Texas the worker size-distribution is much more favorable for *P. curvatus,* and it rapidly reaches densities around 8 times greater than *P. tricuspis* in similar habitats. In August 2007 we will be evaluating the likelihood that these greater densities allow *P. curvatus* to discover most mound disturbances before *P. tricuspis,* thereby, pre-empting *P. tricuspis*'s reproductive opportunities. More generally it is likely that in any environment where there is a strong asymmetry in the relative abundance of two nest-disturbance orienting phorid species, the more common species will deprive the less common species of reproductive opportunities regardless of whether they overlap in the worker size classes they need to reproduce.

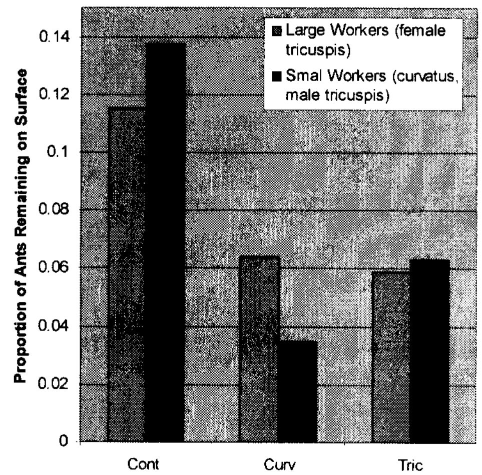

Fig 2: Attack by *P. curvatus* reduces the availability of workers suitable to produce female *P. tricuspis*.

<u>What factors regulate the relative abundance of *Pseudacteon* species?</u>

Does the worker size distribution available at disturbed mounds limit Pseudacteon populations? We initially proposed testing how the size distribution of workers at nest disturbances limits *Pseudacteon* populations. The ability of *P. curvatus* populations (which exploit the more abundant smaller worker caste) to rapidly reach densities 8 times greater than *P. tricuspis* populations in similar habitats indicates that worker-size distribution does in fact impact *Pseudacteon* population densities. However both the finding that *P. curvatus* strongly depresses the densities of *P. tricuspis* in areas where they co-occur, and the finding that these species differ in the contexts in which they locate and attack worker ants, preclude any simple relationship between worker size distribution at disturbed mounds and the relative densities of *Pseudacteon* species. This is particularly true for sites where they co-occur. We only recently have had sites established with only *P. curvatus*, so we have not yet made progress in evaluating the role of worker size distribution in determining single species abundance.

Do P. tricuspis and P. curvatus differ in the contexts in which they exploit S. invicta workers? Observations of competition at baits during the fall of 2006 revealed that in addition to attacking at mounds, *P. curvatus* can be found attacking at up to 50 to 80 percent of baits recruited to by *S. invicta*. However, the frequency with which *P. curvatus* was found attacking at baits was highly variable. Regardless, this is a considerably higher degree of utilization of trails than has been reported previously. This tendency will increase *P. curvatus'* opportunities to find hosts relative to *P. tricuspis*, particularly during hot times of year when mounds are scarce.

To address whether *P. curvatus* and *P. tricuspis* differ in the frequency with which they attack at baits, 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 sampled a first set of 2 sites in August and September of 2006. We plan to finish the sampling in August of 2007. The data indicate that *P. curvatus* attacks *S. invicta* at baits much more commonly than does *P. tricuspis*. At sites in which *P. curvatus* represented 75% of the phorids caught at mound disturbance traps, 98% of the flies caught at bait traps were *P. curvatus*. The frequency of flies at bait traps relative to their frequency at mound disturbance traps does not appear to be influenced by the availability of mounds in the environment, but density of both species appears higher in areas with greater mound availability.

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

We originally proposed to evaluate the efficacy of using midden piles to assess parasitism rates. Our intention was to use the morphology of head capsules hollowed out by phorid larvae to identify ants that had died as a result of parasitism. Phorid specific DNA markers were to be used to validate the identification of parasitized workers.

In the previous funding year, using leveraged funds, we developed phorid specific molecular markers in collaboration with Alexander Mikheyev of the Ulrich Mueller Laboratory. However, our progress on this was slow due to a difficulty finding midden during last summer's drought. Subsequently, a recent paper has undermined the validity of this approach to assessing the mortality induced by parasitism. Henne et al (2007) discovered that parasitized *P. tricuspis* workers do not primarily die in the colony, but leave the colony shortly before pupation. Thus, a large fraction of parasitized workers do not end up in a colony's midden pile. Given this finding, we have suspended our study in its present form but have retained the material for other exploratory work

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

Phorid culturing laboratory

This year saw a major change in culturing operations, as we moved to a mass attack system for rearing phorid flies (Fig 3). The previous system utilized an array of individual attack arenas that will be retained for future experimental use. In the new system, trays containing pupae are placed inside a large climate controlled chamber, and emerging flies then mate and attack ants housed in trays inside this chamber. Ant trays and pupae trays can be switched out as needed. Temperature, humidity and lighting are regulated within each chamber, and a computer is used to automate equipment in the chamber. Five of these mass attack systems have been assembled allowing simultaneous rearing of several species and genetic lineages, along with a decrease in technician time. A detailed review of rearing procedures has allowed reductions in technician time associated with counting pupae numbers and ant care.

Other aspects of the rearing process have also been improved, including use of a pupal incubation chamber allowing pupal development at 95% relative humidity, which is critical for low mortality. Additional alarm sensors and an automated dialup service have been included to ensure integrity of the environmental controls.

Fig 3. Mass attack system in phorid lab. Five chambers are used for high efficiency rearing of phorids.

Total fly production for the year within the mass attack system was 84933 *tricuspis,* 22197 *obtusus* and 21613 *curvatus.* These figures only include adult flies that were cycled through the mass attack system and excludes any of their offspring that were in trays of infected ants returned to the field. At any time, approximately 20 - 25% of the ants are destined for return to the field.

Several experiments have been conducted in the lab to better understand the life histories and development of several phorid species, leading to improved fly production. One experiment looked at the effect on fly population growth when using ants from different localities, but little difference was found in fly performance. However a further experiment on the effects of ant quality showed a strong influence of diet. Here we used several treatments of ant diet and found that some combinations of food supplements supported higher fly production, and this will be important for maintaining high fly population sizes during winter when they traditionally decline.

Another experiment looked at the consequences of altering the schedules of watering cycles at different stages of pupal development. We found that time of emergence can be modified by altering the time of day that pupal trays are watered, and this is useful for manipulating the lab schedules. Of greater importance was a strong improvement in survivorship of *curvatus* when early stage pupae receive less frequent watering while still maintaining humidity at a high level, as suggested by Vogt et al (2003). These experiments provide a better understanding of fly development and are especially important as we look towards bringing additional species into the lab.

Releases

During FY 2006-2007, a major release effort was undertaken using the "trojan" method of digging, infecting and returning colonies. This method proved extremely effective in establishing *P. curvatus* at all 9 sites treated during the 2006 season in south and central Texas, and additional sites are now being added to the *curvatus* release program. A further major development was the successful establishment of *obtusus* at Brazos Bend State Park in late 2006 using the infected colony method, and this species is now being released at additional sites. We are now also using this release method with *tricuspis* at several sites in south Texas. Given the current favorable weather conditions during 2007, there is hope that most of these newly established populations will persist and spread. Earlier releases of *P. tricuspis* begun around 1999, have spread over more than 7 million acres of Central and Coastal Texas, and *P. curvatus* has now also begun an expansion phase from earlier releases in several sites (Gilbert et al, submitted).

In previous years, the standard release method was based on direct releases of adult flies, which has several potential limitations. The adult flies were vulnerable to storage and travel conditions, and local conditions at the release site. In this previous method, adult flies would be directly released onto disturbed mounds under small enclosures, but their motivation to mate and attack was not always high. The "trojan" method allows for attacks on ants under controlled conditions, and the ants may provide better conditions during storage and travel. But a further important element in the success of the "trojan" method is the ability to infect a colony across a longer time frame, typically 5 – 7 days, and thus promote the establishment of overlapping generations. This may be key to establishment success where flies later emerge across a longer period in the field, providing a better buffer against adverse weather conditions.

Our selection of sites includes uses principles of landscape and metapopulation ecology to overcome issues of establishment along the edge of *invicta's* range, where weather extremes may affect continued persistence of fly populations. We select release sites that will potentially form large source populations in favorable areas such as river bottoms and coastal areas, allowing persistence during extreme weather events. Such source populations can then spread along drainage corridors or via long distance wind assisted dispersal to reach remote sites that may not otherwise be suitable for establishment or where flies periodically die out due to extreme weather events.

Table 2. Summary of *Pseudacteon* releases during FY 2006 - 2007. Release effort is measured by then number of colonies that were removed, infected and returned. Shown in bold are the release efforts that were successful and completed, while other releases are still ongoing.

Site	**County**	**tricuspis**	**curvatus**	**Obtusus**
La Paloma	Kenedy		**31**	14
Millett	La Salle	6	**27**	
Brownsville TNC	Cameron		5	
Retama	Webb		7	
La Pryor	Zavala	6	**30**	
Daughtery WMA	McMullen	6	**27**	
Leona River	Zavala	6	**4**	
Brazos Bend SP	Fort Bend		**38**	**10**
Concan	Uvalde	3	**25**	
Pedernales Falls SP	Blanco		**43**	18
Cotulla	La Salle		**13**	
		27	**250**	**42**

In late 2006, we initiated a series of multi-species releases in Kenedy County, designed to replicate a full community of *Pseudacteon* from an arid zone of Santiago del Estero, Argentina. This method is being explored to determine the feasibility of direct releases without first establishing the flies in the laboratory. All species involved have been previously investigated and host specificity tests completed. The acquisition of pupae from Argentina is in collaboration with Dr P Folgarait (Universidad Nacional de Quilmes), and requires a major coordinated effort to secure these stocks. It is too early to assess the outcome of this ongoing release experiment.

Table 3. Flies imported from Argentina. Abbreviated column headings represent *P. nocens, P. obtusus, P. tricuspis, P. curvatus, P. litoralis, P. nudicornis, P. cultellatus.*

Total pupae	**Shipment date**	**noc**	**obt**	**tric**	**curv**	**lit**	**nud**	**cult**	**males**
2792	Dec	3.8%	8.6%	4.8%	11.4%	1.9%			69.5%
415	Feb	5.9%	2.9%	9.8%	7.8%	4.9%			68.6%
2226	April	14.2%	3.4%	4.5%	21.0%	4.5%	0.6%		51.7%
2378	May	4.2%	0.6%	8.9%	22.5%	2.5%		0.8%	60.6%
2202	June	26.4%		1.4%	3.4%	4.3%			64.4%
10013									

Tracking expanding Pseudacteon populations

In the spring of 2006, we undertook a detailed survey of the pattern of expansion in the Central Texas and Gulf coast. The preliminary results of this effort are described in the 2006 Annual Progress Report. This effort was critical to determining optimal study site locations and planning field releases. In this funding cycle, in September through November of 2006, we resampled transects sampled in the spring in order to assess rates of spread, the role of the prevailing wind in determining spread rates, and the dynamics of population establishment at the leading edge of the spreading population. The sampling was accomplished using sticky traps developed in the 2006 annual funding period and described in that report. The results of this work are currently in press at Biological Invasions (LeBrun et al in press). The major findings were that population spread is largely governed by the prevailing wind with population edges going into the wind traveling by local diffusion at a rate of 10 to 14 km per year, and population edges traveling with the prevailing wind combining local diffusion with long-distance, wind mediated effective dispersal to realize spread rates of 45 to 70 km per year (Fig 4). The understanding gained from this work will allow us to predict the likely location of the expanding population front of the various *Pseudacteon* populations outlined in Table 1 based on yearly samples of the appropriate axes relative to the wind.

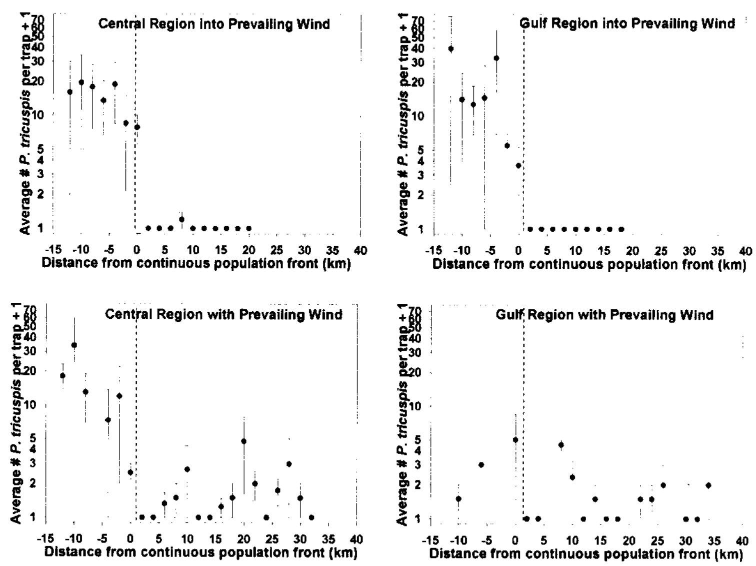

Fig 4: Population edges moving into versus with the prevailing wind. Spread with the wind is dominated by long-distance dispersal with populations founding in front of the continuous front.

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