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Introducing Phorid Fly Parasitoids of Red Imported Fire Ant Workers from South America to Texas: Outcomes Vary by Region and by *Pseudacteon* Species Released

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Since the first report on introductions to Texas of Pseudacteon decapitating flies, a variety of participants have released flies in a range of sites. The expansions of Pseudacteon populations have been systematically and widely monitored. Before 2002, the widely released initial species P. tricuspis Borgmeier Severe drought in 1996-2001 and host-sizedid not become established. dependent sex ratio were proposed constraints in establishing this species. recent years, however, these limitations have been lifted in some areas by favorable weather, irrigation of release sites, and/or by use of a smaller Pseudacteon species, P. curvatus Borgmeier, not reliant on larger fire ant workers to produce females. Beginning in 2002, the USDA-APHIS collaboration with USDA-ARS and Texas Cooperative Extension programs began to supplement release sites in Texas beyond those initiated by the University of Texas, Austin phorid fly project. In 2005, private citizens began to participate in the spread of Pseudacteon to new sites. By fall 2006, P. tricuspis, expanding from releases between 1999 and 2001, was found on more than 3 million hectares of Central and Coastal Texas, while P. curvatus, with its later start, is only now beginning to expand at some sites. Pseudacteon that established more easily in mesic and moderate climates has difficulty surviving unfavorable weather in South Texas. However, two sites where flies "failed" to become established were revealed to be false negatives after the record rains of summer 2007. Starting in late 2006, the first releases of P. obtusus Borgmeier in North America established, and three to five additional species are being released.

**Resumen.** Desde el primer reporte del estado de las introducciones en Texas de la mosca decapitadota *Pseudacteon* ha habido avances significativos en las actividades de liberación por una gran variedad de participantes. Un monitoreo sistemático de la expansión de las poblaciones de *Pseudacteon* se encuentra en

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Antes del 2002, establecimientos exitosos de una especies ampliamente liberada, P. tricuspis Borgmeier, fueron bastante pobres. factores, una severa seguía entre 1996-2001, y el radio del sexo dependiente del tamaño del hospedero se propusieron como problemas en el establecimiento de esta especie. Sin embargo, en años recientes, estas limitaciones han sido cambiadas en algunas zonas por periodos de clima favorable, irrigación de sitios de liberación, y/o por el uso de una especie mas pequeña de Pseudacteon, P. curvatus Borgmeier, no restringido a producir hembras en obreras de largo tamaño. Al inicio del 2002, el USDA-APHIS en colaboración con el USDA-ARS y el Servicio de Extension de Texas, comenzaron a suplementar liberaciones en Texas por encima de esos iniciados por el proyecto de moscas fóridos de UT-Austin. En el 2005, se comienza la participación de ciudadanos regulares en los esfuerzos de liberación de Pseudacteon en nuevas áreas. Para el otoño del 2006, P. tricuspis, se expandió desde los puntos de liberación conducidos durante 1999 y 2001, la cual se puede encontrar en un área alrededor de 7 millones de acres en el Centro y la Costa de Texas mientras que P. curvatus, con su liberación mas reciente, se encuentra en fase de expansión en varios sitios. El sur de Texas continua presentando problemas con poblaciones de Pseudacteon que se han establecido con mas facilidad en climas mésicos y moderados. Sin embargo, dos sitios que "fracasaron" recientemente revelaron ser falsos negativos después de registrarse lluvias record durante el verano del 2007. Casi al finalizar el 2006, primeras liberaciones de P. obtusus Borgmeier en Norte America han resultado en establecimientos y liberaciones en 3-5 sitios adicionales se encuentran en desarrollo.

#### Introduction

The purpose of this communiqué is to update the status of efforts to establish South American phorid flies in Texas as part of broader efforts to control the red imported fire ant, Solenopsis invicta Buren. It is still too early to demonstrate impacts of introduced flies on suppressing fire ants (Morrison and Porter 2005). The background and early phases of phorid introductions in Texas were reviewed by Gilbert and Patrock (2002). Subsequently, widespread advances have been reported in establishing Pseudacteon species across the southern US (Graham et al. 2003, Porter et al. 2004, Thread et al. 2005, Pereira and Porter 2006, Vazquez et al. 2006). Most of these projects have been enabled by cooperative "areawide" programs involving USDA-APHIS, USDA-ARS and extension programs (Pereira 2003). Most release sites in Texas have been established since late 1995 by the fire ant/phorid research program at Brackenridge Field Laboratory of the University of Texas at Austin. These efforts were focused primarily south of a line from Mason through Austin to Galveston. The localities of these releases were selected because of logistics, opportunity and an interest in testing S. invicta habitats different from those of the more mesic southeastern states. Flies have been released at additional sites primarily east and north of Austin, through a USDA-APHIS release program coordinated by participants employed by Texas Cooperative Extension and Texas A&M University several of whom are coauthors of this report. All have coordinated efforts to avoid initiating releases near zones with flies already established, to maintain samples for later genetic tracking and to avoid transient "fly-free zones" currently used as comparison control sites for long-term evaluation of the impact of Pseudacteon on fire ants.

### Highlights of Developments, Changes and Outcomes of the Pseudacteon Release Program in Texas since November 2001

With regard to the extent of spread by naturalized *Pseudacteon* populations in Texas, this review period covers establishment of flies between fall 2001 and fall 2006. However, with regard to the fate of local releases, updates through July 2007 are included. Initial efforts in Texas are summarized by Gilbert and Patrock (2002). Developments in the phorid release program during this review period include:

- 1. Rains in July 2002 and through the summer in central Texas ended a long drought and led to the first conspicuous increase in abundance of P. tricuspis since its establishment in 1999 at Brackenridge Field Laboratory. Flies from a new areawide introduction near Caldwell in spring 2002 established quickly the following year. Flies at Austin and Caldwell expanded into new areas through 2003, as many as 13 km in the former case.
- 2. All attempts to establish P. tricuspis south of San Antonio (N = 7) seemed to have failed by fall 2004, including four sites where flies had established for a short period (shown in Fig. 2 in Gilbert and Patrock 2002). All of these populations were introduced in areas with abundant S. invicta populations near impounded drainages (cattle tanks). At Retama Ranch's "Big Lake" site in Webb County, P. tricuspis persisted for more than two years (May 1999-September 2002) of mainly drought months, as ants moved colonies and mounds to stay near moist borders of a drying cattle tank. By 13 August 2001, the lake at the release site was almost dry as north-facing and east-facing photos from the tank dam show (Fig. 1. top, left and right). The dotted line in



Fig. 1. Successive drought and flood events hindered persistence of nascent *Pseudacteon* populations at Big Lake, Retama Ranch, Webb County, TX (see text).

the top right photo shows the edge of the red imported fire ant colonies at that time, all clustered a few meters from that boundary. When intense "tank rains" raised water levels ~7 m virtually overnight (fall 2002), fire ant colonies floated to the new waterline and phorids disappeared at the site. Additional heavy rains in fall 2003, carried water around the dam, floating red imported fire ant colonies to areas previously not occupied. The lower two photos in Fig. 1 show the same north and east views on 21 December 2003 from slightly different vantage points. The rains came too late to save large willow trees on the tank dam that perished in 2002 (compare top left and bottom left). The "X" is a reference point for relating the 2001 and 2003 photos. Because fire ants are scarce in arid South Texas, being primarily concentrated near drainages and impounded water, we believe this drought-flood cycle is likely to reduce the potential of many such sites to function as source populations for introduced phorids. Other sites where flies established briefly before 2001 might have failed under similar scenarios, including sites near Millett (LaSalle County), Welder Wildlife Area (Aransas County) and Escondido Lake, King Ranch (Kleberg County). Because of drought during attempted establishment, flies did not become abundant, and until unusually rainy conditions in summer 2007, none were known to have expanded beyond the immediate release areas before becoming extinct (see Section 6).

- Additional specificity data for P. curvatus (Porter and Gilbert 2004, Vazquez et al. 2004. Vazquez and Porter 2005) convinced us to shift an earlier position (Gilbert and Patrock 2002) against use of this species in Texas based on initial sequential no-choice trials. P. curvatus was released near Caldwell and at Brackenridge Field Laboratory in 2004. This species rapidly established at both sites, exceeding abundance of P. tricuspis 10 fold by spring 2005. P. curvatus. like P. tricuspis, is a species that orients principally to disturbed mounds rather than foraging trails (Orr et al. 1997); however, it is a smaller species, and not limited by worker size to produce females (e.g., Morrison and Gilbert 1998).
- With abundant P. curvatus in the field and limited production in the laboratory, the University of Texas group initiated a program of introducing phorids to new sites by digging and separating colonies at the experimental site for later attack in laboratory chambers or in established field sites near Austin. "Trojan colonies" were returned to home mounds a few days later. Consequently, since 2005, colonies of P. curvatus at Brackenridge Field Laboratory have been the source of successfully introduced populations at approximately 10 additional sites across the focal region of the University of Texas group. In northeastern Texas, four additional sites for P. curvatus have been initiated from infected ants sent to Texas A&M University from the USDA-APHIS-funded rearing center in Gainesville, FL. These ants (approximately 2 g) were collected from mounds on these locations and sent to the rearing facility where ants were exposed to flies for 48 hours and returned to Texas for release. Ants were released to the original mounds from where they were collected.
- In early summer 2005, the University of Texas Laboratory assisted the Bee County Wildlife Management Association in applying the 'Trojan colony' method in an attempt to introduce both P. tricuspis and P. curvatus from Austin to two private ranches in that county (near Mineral around a cattle tank and near Beeville around a cattle tank and an irrigated garden). A record-setting drought occurred in Bee County during the next year and no phorids established around

either cattle tank site. However, P. curvatus established in the irrigated patch

and spread after rains returned.

The difficulty of knowing the outcome of release efforts is a function of manpower and time required to monitor the presence of flies, compounded by the large number of sites. At low densities, Pseudacteon is difficult to monitor effectively by direct observation. Thus, one of us patrolled and watched ten disturbed mounds for three hours to see a single male P. tricuspis, the first evidence of establishment at a release site in Wharton County where P. tricuspis now is widespread. Several methods were developed to improve detection of Pseudacteon. For example, Barr and Calixto (1996) used electrical stimulation by a modified livestock prod to detect flies. More recently, Puckett et al. (2007) and E. LeBrun developed two similar phorid-trapping methods (sticky traps) that have improved the efficiency of monitoring phorids, reducing the chance of false negatives and allowing large areas to be assessed for relative abundance and limit of spread. Such methods document that P. tricuspis, spreading from early releases initiated by the University of Texas group in Travis, Wharton and Brazoria counties, now is found on approximately 3 million hectares of Texas (LeBrun et al. 2007 in press) and that P. curvatus, with its later start, is rapidly spreading over the same regions (Figs. 2 and 3). Similar studies have found flies spread from the USDA areawide sites at Caldwell and Vidor. Most recently, following the wet conditions of spring 2007, we found our southernmost release of P. tricuspis in Cameron County, thought to be extinct since fall 2003, is thriving on The Nature Conservancy's Southmost Preserve. Trap monitoring in July 2007 showed P. tricuspis now extends at least 12 km northwest from the original release site. unexpected result suggests the southernmost introduced P. tricuspis flies are widespread along the Rio Grande corridor. This must include the Mexican side of the border because the greatest concentration of P. tricuspis is less than 200 m from the opposite bank of the Rio Grande. This example points to the issue of false negatives resulting from infrequent monitoring and/or monitoring during hot, dry intervals such as have been frequent in Texas during these activities.

Results of host specificity studies were published and interpreted for several additional species of Pseudacteon (Porter and Gilbert 2004, Estrada et al. 2006). Porter and Gilbert tried to obtain permits for release of these additional species in fall 2006. USDA-APHIS ruled that because Pseudacteon phorid flies are not plant pests, they (APHIS) did not assume jurisdiction over the question of outdoor release of exotic species of this genus and thus assigned that authority to appropriate federal and state officials (letter dated 11 August 2006). With permission from the Texas Department of Agriculture, the phorid laboratory at Brackenridge Field Laboratory began releasing P. obtusus at three sites (Table 3), with establishment at Brazos Bend State Park (Ft. Bend County). In late 2006, the Brackenridge Field Laboratory 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 community, each species of which has been studied, includes P. litoralis, P. nocens, P. obtusus, P cultellatus, P. nudicornis as well as P. tricuspis and P. curvatus. It is too early to assess the outcome of this ongoing

release experiment.
A summary of *Pseudacteon* releases in Texas and their current status are

summarized in Tables 1, 2, and 3 and Figs. 2 and 3.

Table 1. Texas Release Sites for P. tricuspis. The methods of infection and current status are shown. Areawide releases USDA/TCE) are from phorid stocks from Brazilian (near Rio Claro, Sao Paulo) P. tricuspis introduced by S. Porter and produced in he ARS Gainesville facility. UT stocks include Brazilian lines from Campinas SP and Rio Claro SP via Porter and Formosa, Argentina discontinued by Porter but maintained at the University of Texas rearing facility). Lines introduced at each site (not reported here) are ecorded for later interpretation of results. Many sites represent mosaics of lines and higher genetic variance, thus maximizing potential for evolutionary adjustment to the diverse habitats of Texas.

		Release	Flies	Mounds		
County	Release site	period	released	infected	Agency	Outcome
Bastrop		4/99	5,270		UT, USDA-ARS, SHSU	failed
Bastrop		12/99 - 4/02	16,642		15	established 8/02
Bee		9/02		80	5	failed
Bee		6/05		8	15	failed
Bexar		12/05	7,530		<b>USDA-APHIS, TCE</b>	no detection 4/06 or 9/06
Brazoria		1/01 - 6/04	6,212		5	established 6/04
Burleson		4/02, 5/03	5,587		USDA-ARS, TCE	30 km by 9/06
Cameron		11/02 - 3/03	12,637		T)	12 km by 6/07
Colorado		7/02 - 6/03	15,647		UT,TCE	failed
Denton		10/06	2,906	53	TCE, TPWD	established 10/06
Denton		10/02, 5/03	5,756		<b>USDA-APHIS, TCE</b>	failed
Dimmit		4/05 - 6/05	1,708		5	failed
Hays		6/02 - 10/02	3,825		5	failed
Kenedy		8/05 - 12/05	2,668	80	5	6 km by 7/07
Kenedy		12/99 - 3/02	3,224		5	failed
Kerr		90/90	>1,000		5	established 09/05
Kleberg		12/99 - 3/02	6,406		5	lasted 37 weeks
La Salle		8/00 - 4/05	19,992		5	lasted 58 weeks
Lamar		5/05, 7/05	2,000		SHSU	established 06
Fee		6/02 - 9/02	12,015		5	failed
McLennan		6/07 - 7/07	029	26	TCE	ongoing release
Orange	Vidor	4/02	1,939		TCE, UT, USDA-APHIS	30 km by 1/06
Polk		2/02	u/k		USDA-APHIS, TCE	established 05

established 3/00 asted 112 weeks unknown source established 7/00 established 8/03 recolonized from established 9/03 established 6/01 established 5/01 established 5/07 established 04 Outcome failed failed failed failed failed failed failed USDA-APHIS, TCE Agency Mounds nfected 9 1.920 eleased 25,696 33,250 8,315 4,113 26,549 2,862 16,663 6,751 5,017 18,445 9,081 6/99 - 11/99 9/00 - 10/021/95 - 2/99 2/99 - 6/00 1/02 - 1/03 1/99 - 1/00 2/00 - 6/00 00/9 - 66/1 2/00 - 4/00 4/06 - 7/06 1/99 - 4/01 1/04 - 3/04 5/00 - 4/03 Release period 90/8 5/04 Retama Big Lake Retama RH Tank BFL before 1999 Retama S Tank N of Livingston Release site Samp Mabry Onion Creek Indiangrass St Edwards Hungerford Horsethief **BFL 1999 Batesville** Huntsville La Pryor Welder San Patricio Table 1. cont. Wharton County Zavala Zavala **Fravis** Travis Travis **Fravis** Webb Webb Travis **Travis** ravis Webb Polk

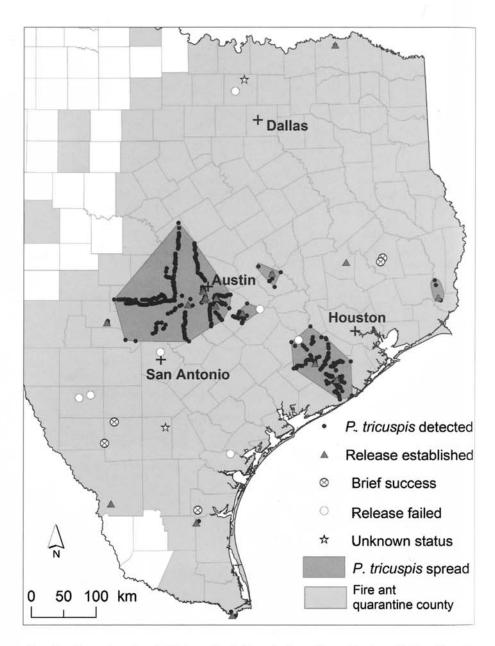


Fig. 2. Spread and establishment of *Pseudacteon tricuspis* decapitating flies in Texas as determined by different trap methods.

Table 2. Texas Release Sites for P. curvatus. The methods of infection and current status are shown. Ultimate origin of phorids is Corrientes, Argentina, S. Porter, USDA. Proximate origin is the ARS rearing facility in Mississippi for areawide releases and outdoor population at Brackenridge Field Laboratory for all sites initiated by the University of Texas.

		Release	Flies			
County	Release site	period	released	Mounds infected	Agency	Outcome
Bastrop	Stengl	2005	1,210		TD TD	established 7/07
	Beeville	2/05		80	5	2 km by 5/07
	Pedernales Falls SP	2006		24	5	established 5/07
	Brazos Bend SP	2006	5.00	37	5	established 4/07
	Cole Ranch	2005	615		5	established 5/06
Burleson	Caldwell	4/04		139	USDA-ARS, TCE	30 km by 9/06
	Brownsville	2007		9	5	ongoing release
	New Braunfels	90/9		36	USDA-APHIS, TCE	unknown
	Denton-Wise Co.	9/04		63	USDA-APHIS, TCE	10 km by 09/06
	La Paloma	2006		47	5	3 km by 5/07
	Coolwater	2005	1,021		5	10 km by 6/07
	Cotulla	2007		12	5	ongoing release
	Millett-Falsette	2006		29	5	established 4/07
	Daughtery WMA	2006		25	5	established 4/07
	BFL	2004	971	2	5	24 km by 5/07
	Horsethief	2005	862		5	established 5/06
	Indiangrass	2005 - 06	1,816		5	established 5/06
	Concan	2006 - 07		22	5	established 6/07
	Huntsville	9/05		17	USDA-APHIS, SHSU, TCE	established 06
	Retama Big Lake	2006 - 07		7	5	established 7/07
	Batesville	2006		00	5	established 06
	La Pryor	2005	77	37	5	established 06

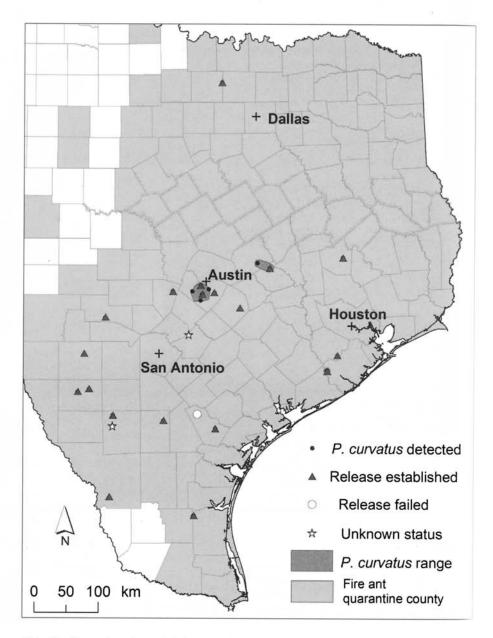


Fig. 3. Spread and establishment of *Pseudacteon curvatus* decapitating flies in Texas as determined by different trap methods.

Table 3. Texas Rlease Sites for *P. obtusus*. The methods of infection and current status are shown. Ultimate origin of phorids is Herradura, Formosa, Argentina (October 2002). Proximate origin of releases in Texas is the *obtusus* lineage transferred to Brackenridge Field Laboratory for rearing and research purposes from USDA-ARS stocks in Gainesville, FL.

County	Release site	Release period	Flies released	Mounds infected	Agency	Outcome
Blanco	Pedernales Falls SP	10/06 -		9	UT	ongoing release
Brazoria	Brazos Bend SP	8-11/06		11	UT	established 06
Kenedy	La Paloma	6/07 -		9	UT	ongoing release

#### Discussion

Results since early 2002 allow us to retrospectively judge the assertions and conclusions in our prior summary of Pseudacteon introductions in Texas. First, our earlier reluctance to release P. curvatus was based on initial laboratory tests of specificity (it was the least specific of any species tested in sequential no-choice tests) and on the fact that in Texas, invading S. invicta and native fire ants often coexist, at least briefly, in a mosaic of sharply delimited patches. Initially, we were concerned that the tendency (in laboratory tests) of P. curvatus to attack S. geminata-group species while in an attack mode after stimulation by exposure to S. invicta would be a negative factor for the native ants even if the fly did not complete development. This concern seemed justified given the possibility that observations in the laboratory would translate to the field and attacks on natives by P. curvatus would cause competitive turnover at food sources, e.g., Orr et al. (1995). We believe our conservative approach was justified and our delay in initiating releases of P. curvatus pending additional data (Porter and Gilbert 2004, Vazquez et al. 2004, Vazquez and Porter 2005) will make little difference. A graphic illustration of the specificity of P. curvatus in the field is shown by Fig. 4, in which S. invicta was under attack in one tray in July 2005, at Brackenridge Field Laboratory while S. geminata in an adjacent tray was ignored. Additional evidence of the specificity of P. curvatus comes from traps baited with S. geminata workers in an area with P. curvatus. Such traps capture S. geminata phorids (P. bifidus, P. spatulatus, P. browni), but not P. curvatus (E. LeBrun, unpublished data). Finally, it is clear now that Pseudacteon species differ greatly in their effects on attacked workers (Wuellner et al. 2002), including effects on foragers. Mound-specialist phorids such as P. tricuspis and P. curvatus are unlikely to impact foraging efficiency since they rarely recruit to food being harvested at the end of foraging trails unless there is also a disturbance of the foraging ants (Orr et al. 1997, Morrison and King 2004).

Results of releases since 2002 generally support the ideas that the occurrence of drought and predominance of polygyne fire ants (and this form's relative lack of larger workers when compared to the monogyne form that hosts *P. tricuspis* in South America) separately or as interacting factors may constrain success in introducing *P. tricuspis*. We noted that the return of regular rains after dry periods seemed to have been associated with establishment and expansion of

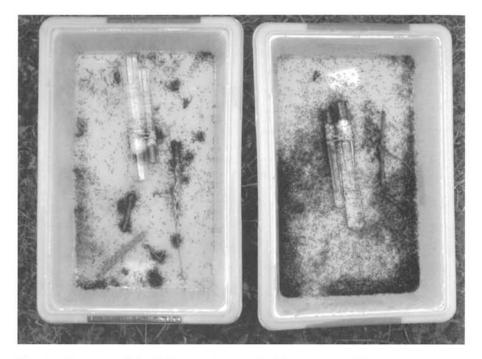


Fig. 4. Host-specificity field experiment with *P. curvatus*. Trays containing *S. invicta* (left) and *S. geminata* (right) were set out for attack by a naturalized population of *P. curvatus* at Brackenridge Field Laboratory. *P. curvatus* only attacked *S. invicta* as evidenced by the strong defensive behavior of the fire ants, which retreated to clumps. *Solenopsis geminata* shows this kind of defensive behavior when attacked by its own *Pseudacteon*.

*P. tricuspis* and *P. curvatus* at several sites. Moreover, the success of using an irrigated release site during drought in Bee County has become standard practice in the arid South Texas zone. A more formal analysis of rainfall patterns and establishment success is one goal of future research.

That *P. tricuspis* managed to survive as a meta-population in two areas of South Texas where monitoring indicated failure of establishment shows how little we know of the ecological requirements and spatial ecology of these insects. New trapping techniques lower the threshold at which phorids can be detected. Even so, phorids are able to resist detection when scarce, especially if the release site is a sink rather than a source under most prevailing weather conditions.

Evidence that worker size distributions constrain establishment and spread of *P. tricuspis* because of its reliance on larger workers for production of females (Morrison and Gilbert 1999) is indirect. Numerous seemingly suitable sites in terms of abiotic conditions and mound densities proved unsuitable despite large numbers of this phorid being released. Also, the smaller *P. curvatus*, not reliant on larger ants, generally establishes more readily and increases rapidly in abundance where both species overlap. We hypothesize that drought and availability of larger worker sizes in *S. invicta* interact such that colonies under nutritional stress during drought have reduced capacity to regenerate their worker force and larger workers are more

likely to be lost without replacement. Not only are *S. invicta* workers less available to phorids on the soil surface during drought, under this hypothesis the ants are of lower quality for sustaining *P. tricuspis* populations. An alternate hypothesis is that drought conditions result in reduced plant cover and warmer soil temperature, thus decreasing the number of phorid pupae that survive. Many factors influence the success of establishment and the dynamics of spread by *Pseudacteon*, and Texas, with its temporal and spatial diversity of climate and habitat, interacting with seasonal effects, will be a laboratory for studying such factors. Our work provides quidance to the factors that must be considered and tracked as this effort proceeds.

Future directions for research and management include 1) introduction of numerous phorid species to cover a range of niches, 2) monitoring the spread of each species of phorid across a range of ecoregions for planning effective release strategies, 3) monitoring the dynamics and interactions of these newly assembled phorid communities, and 4) evaluating the impacts of these suites of introduced phorids on fire ants. The time scale for this program is constrained by the 1) rate at which new species can be cultured and established, 2) lag time encountered during the establishment and subsequent spread of the phorids, 3) response time of fire ant colonies to potential impacts by phorids through changes in resource allocation and impacts on long-term colony health, and 4) time needed for native ant communities to respond. Constraints 1 and 2 are conditioned by the likelihood of encountering favorable weather when opportunities are available to acquire and release species of phorids.

During the past five years we have learned more about the life histories and ecology of *Pseudacteon* parasitoids of *Solenopsis*. These studies have reinforced the goal stated in Gilbert and Patrock (2002) to eventually introduce an entire suite of complementary *Pseudacteon* species to include not only mound-orienting species but those that orient to foraging trails and influence interference competition between *S. invicta* and other species. As previously proposed, *Pseudacteon* from source sites that match some of the extreme habitats colonized by *S. invicta* in Texas may be required to fully test the potential of phorid flies to reduce the dominance and pest status of *S. invicta* in our region.

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