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## CHAPTER 13. ASSESSING HOST SPECIFICITY AND FIELD RELEASE POTENTIAL OF FIRE ANT DECAPITATING FLIES (PHORIDAE: *PSEUDACTEON*)

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### BACKGROUND OF SYSTEM

Fire ant populations in their South American homeland are about 1/5 to 1/10 as dense as populations in North America (Porter *et al.*, 1992; Porter *et al.*, 1997a). This intercontinental difference in fire ant densities was not explained by differences in climate, habitat, soil type, land use, plant cover, or sampling protocols (Porter *et al.*, 1997a). Escape from numerous natural enemies left behind in South America is the most apparent explanation for the intercontinental population differences. Natural enemies left behind in South America include two species of microsporidian pathogens, three species of nematodes, about 20 species of phorid decapitating flies, a eucharitid wasp, a parasitic ant, and numerous other microbes and arthropods of uncertain relationship to fire ants (Porter *et al.*, 1997a). Escape from coevolved ant communities may also have been important. Ants in Brazil and Argentina, however, do not appear to be any more abundant than those in the United States, at least as indicated by their ability to find and occupy baits (Porter *et al.*, 1997a).

Classical or self-sustaining biological control agents are currently the only potential means for achieving permanent regional control of fire ants. Poison baits can effectively control fire ants in high value areas (Drees *et al.*, 1996; Williams *et al.*, 2001), but they are too expensive for use in rangeland and are not sufficiently specific for use in natural areas. Once initiated, baits must be reapplied two to three times each year in perpetuity, or the fire ants will return – often in even higher densities because competing ants have also been eliminated. Successful use of biological control agents will not eradicate imported fire ants, but it could help shift the eco-

logical balance in favor of native ants. If this happened, fire ant populations in the United States could be reduced to levels similar to those in South America (Porter, 1998a).

#### SIMILARITY TO WEED BIOLOGICAL CONTROL

Fire ant colonies are like perennial plants in at least four ways: (1) their relative immobility, (2) their longevity, (3) the two to three years required to reach reproductive maturity, and (4) the fact that resource gathering depends on non-reproducing components (sterile workers as compared to roots and leaves). Not surprisingly, biological control of fire ants is more like exotic weed biological control than standard insect biological control. First, unlike pest populations on many agricultural crops, fire ant populations do not rise and fall dramatically in a period of a few weeks (Tschinkel, 1993). Consequently, fire ant biological control agents do not need high growth rates in order to suppress fire ant populations; their populations simply need to increase gradually until they are effective. Second, fire ant biological control agents do not need to kill their host to be effective. They only need to stress their hosts so that they have difficulty competing with other ants, just as biological control agents of weeds only need to stress their hosts so the target weeds can no longer compete with other plants in the community. Biological control of agricultural pests such as aphids, in contrast, could not rely on competition from other species of aphids to be effective as these would also be crop pests. A third similarity with weed biological control is that particular biological control agents may only be effective in certain habitats, creating a control mosaic in which single agents affect anywhere from a small fraction of all infested sites to most of the landscape. This produces a situation in which control is often best obtained by introducing a small community of natural enemies that are effective under various environmental conditions. (In contrast, crop monocultures make pest control by one or a few natural enemies more likely.) A fourth similarity is that fire ants are landscape pests that affect huge contiguous areas. While chemical control and augmentative biological control are usually cost-effective only when used on limited areas of high value, classical biological control agents are most cost effective when infested areas are large.

#### DESCRIPTION OF PEST INVASION

The black imported fire ant, *Solenopsis richteri* Forel, was inadvertently introduced into the United States at the port of Mobile, Alabama, around 1918 (Loding, 1929). The red imported fire ant, *Solenopsis invicta* Burden, was introduced into the same port some time during the 1930s (Lennartz, 1973). The red fire ant was by far the more successful of the two invaders. It currently occupies over 300 million acres in 12 southern states from Texas to Virginia (Callcott and Collins, 1996, USDA-APHIS, 2004). Strict quarantine procedures have significantly limited the spread of this pest (Lockley and Collins, 1990), but it has nevertheless become established in California, Australia, and across much of the Caribbean (Davis *et al.*, 2001; Nattrass and Vanderwoude, 2001; Jetter *et al.*, 2002). Unless checked, this pest has the potential to occupy tropical and warm temperate regions around the globe (Morrison *et al.*, 2004).

After its introduction in Mobile, Alabama, the black imported fire ant was driven northward by competition from the red imported fire ant and currently is restricted to a small region around the tri-state border of Mississippi, Alabama and Tennessee. However, a broad band of hybridization between red and black fire ants extends from the Mississippi River through to

Atlanta, Georgia (Shoemaker *et al.*, 1996). Red and black fire ants, however, are still considered separate species because hybridization apparently does not occur in native Argentine populations (Ross and Trager, 1990). Because black and hybrid fire ants do not occupy major ports in the United States, their opportunities for further dispersal are greatly limited. Nevertheless, dispersal out of the port of Buenos Aires in Argentina still remains a possibility.

#### DESCRIPTION OF THE PROBLEM

The major problem with invasive fire ants is that there are so many of them. In north Florida pastures, fire ant densities average 1,800 ants per square meter in single-queen areas and 3,500 ants per square meter in multiple-queen areas; this works out to be 15-28 kg/ha or 4-8 tons of fire ants per square mile (Macom and Porter, 1996). Economic damage in the United States is estimated at nearly 6 billion dollars per year (Lard *et al.*, 2001; Pereira *et al.*, 2002), not including environmental damage. Damage from imported fire ants can be grouped into four major categories: agricultural, electrical, medical, and environmental. Imported fire ants adversely affect several important agricultural crops, including soybeans, corn, potatoes, and citrus (Adams, 1986; Adams *et al.*, 1988; Banks *et al.*, 1991; Drees *et al.*, 1992). Fire ants are also known to prey on many beneficial insects including some biological control agents (Eubanks, 2001). Imported fire ants are also a major source of electrical problems: transformers, air conditioners, traffic switch boxes, airport lights, and other electrical equipment located on the ground are all susceptible to problems caused by fire ants chewing off insulation, jamming switches, or building mounds in electrical boxes (MacKay and Vinson, 1990; Vinson and MacKay, 1990). Medical problems from stings are the third major category of problems associated with fire ants. Young children are commonly stung dozens to hundreds of times when they stand on fire ant mounds; several people die each year from fire ant stings – mostly bedridden patients in nursing homes or people who are unconscious or otherwise unable to respond to the fire ants. About 1-2% of the population are sensitive or allergic to fire ant stings (Vinson, 1997). Environmental damage is also associated with imported fire ants. High densities of fire ants displace most native ants from open habitats (Porter and Savignano, 1990; Wojcik, 1994), especially in areas disturbed by urbanization, agriculture, or grazing. Deer, mice, shore birds, quail, and lizards are among the vertebrates that can be harmed by high fire ant populations (Allen *et al.*, 1998; Williams *et al.*, 2003).

#### DESCRIPTION OF AGENTS RELEASED OR PROPOSED FOR INTRODUCTION

Given the broad distribution of fire ants in North America and the magnitude of their impact, biological control appears to be most likely to be obtained by release of a suite of natural enemies. The hope is that each new self-sustaining agent will increase the magnitude and breadth of the impact on fire ant populations.

Three types of organisms are being or have been evaluated for release in the United States. Two species of microsporidians are being intensively studied: *Thelohania invictae* Knell, Allen and Hazard and *Vairimorpha solenopsae* Jouvenaz and Ellis. These pathogens slowly kill fire ant colonies in the laboratory and probably also do so in the field (Briano *et al.*, 1995; Williams *et al.*, 1999). Both diseases appear to be host specific (Briano *et al.*, 2002a). An effort will be made to obtain approval for release of one or both of these diseases from quarantine for field

release trials in 2005. The parasitic ant *Solenopsis daguerrei* (Santschi) has also been evaluated as a possible biological control agent for this pest (Calcaterra *et al.*, 1999). However, so far mass rearing and transfer to red imported fire ant colonies in the United States has not been achieved (Briano *et al.*, 2002b). Phorid decapitating flies of the genus *Pseudacteon* are the third group of organisms that are being evaluated for fire ant biological control. The remainder of this chapter will discuss the biology of these flies and detail the process of studying their host specificity and evaluating risks and benefits of their field release.

Three species of South American decapitating flies have been released in the United States. The first species was *Pseudacteon tricuspis* Borgmeier in Texas (Gilbert and Patrock, 2002) and Florida (Porter *et al.*, 1999). This fly attacks medium to medium-large fire ants and is especially abundant in the fall. This species (from near Campinas, Brazil) is well established in eight states. Flies released in Florida and Alabama have spread at least 50-130 km from their release sites (Porter *et al.*, 2004). A three-year study in north Florida, however, failed to detect measurable impacts on fire ant populations (Morrison and Porter, unpubl. data); effects were apparently lower than 10-30% reduction, which was the sensitivity level of this study. A second biotype of this species from northern Argentina has been released at several sites in Texas along with the first biotype, but its establishment, while likely, still needs to be confirmed by biochemical markers. Two biotypes of *Pseudacteon curvatus* Borgmeier have also been established in the United States, one on black and hybrid fire ants in Alabama and Mississippi (Graham *et al.*, 2003; Vogt and Streett, 2003) and the other on red fire ants in Florida (Vazquez and Porter, unpubl. data) and South Carolina (Davis, Pereira and Horton, unpubl. data). This fly only attacks small fire ants and is especially abundant in the late summer. Impacts of this fly have yet to be assessed, but this fly often occurs in higher densities than *P. tricuspis*. A third species of decapitating fly, *Pseudacteon litoralis* Borgmeier, has been released at two sites in north Florida (in July and September 2003). First generation flies were recovered, but establishment has not been confirmed. This fly attacks medium-large to large fire ants and is most active in the morning and late afternoon until dark. A fourth species of decapitating fly, *Pseudacteon obtusus* Borgmeier, is being held in quarantine until permits can be obtained for its field release. Several additional species of decapitating flies are currently in quarantine in Florida and Texas, where attempts are being made to culture and evaluate them for field release. There is a consensus among phorid researchers that locally diverse communities of decapitating flies will provide more effective biological control than a single species because they will attack a broader range of fire ant sizes, in more habitats, during a broader portion of the day, and during a broader portion of the year (Morrison and Gilbert 1998; Gilbert and Patrock, 2002; Mehdiabadi and Gilbert, 2002; Folgarait *et al.*, 2003).

#### LIFE HISTORY OF *PSEUDACTEON* FLIES

At least 20 species of *Pseudacteon* flies have been found attacking fire ants in South America (Figure 1) (Porter and Pesquero, 2001; Brown *et al.*, 2003, unpubl. data). Up to nine species of these flies have been found at a single site (Calcaterra *et al.*, unpubl.). Each species has a distinctively shaped ovipositor that is presumably used in a lock-and-key fashion to lay eggs in a particular part of its host's body. These flies appear to be common and active throughout most of the year, but different species are sometimes more active at different times of the day (Pesquero



*et al.*, 1996) and during different seasons (Fowler *et al.*, 1995a; Folgarait *et al.*, 2003). Most species are broadly distributed (Borgmeier, 1969; Borgmeier and Prado, 1975) across a wide range of habitats and climates (Folgarait *et al.*, 2004).



Figure 1. Female *Pseudacteon* decapitating fly preparing to attack a fire ant worker (left); eight of about 20 species of decapitating flies known to attack fire ants in South America (right). Photos: S. D. Porter. (UGA1295007)

Female *Pseudacteon* flies usually contain a hundred or more eggs (Zacaro and Porter, 2003). During oviposition, one egg is rapidly injected into the ant thorax with a short hypodermic shaped ovipositor. Shortly after hatching, maggots of *Pseudacteon* flies move into the heads of their hosts, where they develop slowly for two to three weeks (Porter *et al.*, 1995a). Just prior to pupation, the third instar maggot appears to release an enzyme that dissolves the membranes holding the exoskeleton together. The maggot then proceeds to consume the entire contents of the ant's head, a process that usually results in rapid decapitation of the living host. The headless body is usually left with its legs still twitching (Figure 2). Worker ants apparently carry the larva-infested head capsule outside their nest to above- or below-ground refuse piles several hours after the host is killed. The maggot then uses hydraulic extensions to push the ant's mouth parts aside, after which it pupates within the empty head capsule, positioned so that the anterior three segments harden to form a plate that precisely fills the ant's oral cavity (Porter, 1998a). The rest of the puparium remains unsclerotized and is protected by the ant's head capsule, which functions as a pupal case (Figure 2). Pupal development requires two to



Figure 2. Fire ant worker decapitated by a *Pseudacteon* fly maggot just prior to pupariation (left); *Pseudacteon* puparium removed from fire ant head (right). Photos: S. D. Porter. (UGA1295008)

three weeks depending on temperature. Adult flies are generally mature and ready to mate and oviposit about three hours after emergence. Based on laboratory observations, adult *Pseudacteon* flies may live up to a week in nature; however, high rates of activity associated with oviposition will shorten their lives to one to three days.

During attacks, fire ant workers are keenly aware of the presence of phorid flies. A single female fly usually stops or greatly reduces the foraging efforts of hundreds of fire ant workers in only a minute or two (Porter *et al.*, 1995b). As soon as a fly appears, most workers rapidly retreat into exit holes or find cover. Other workers curl into a stereotypical c-shaped posture (Porter, 1998a; Wuellner *et al.*, 2002a), a behavior not seen except when the ants are under attack by phorids. Some fly species inhibit fire ant foraging as long as they are present, often for periods of several hours (Folgarait and Gilbert, 1999; Wuellner *et al.*, 2002b). Reduced foraging activity appears to facilitate competition from ants that might otherwise be excluded from food sources in fire ant territories (Feener, 1981; Orr *et al.*, 1995; Morrison, 1999; Mehdiabadi and Gilbert, 2002). Several flies are sufficient to stop nest construction or freeze the activity of entire colonies in laboratory nest trays (Porter *et al.*, 1995b). The overall impact of these flies on fire ant populations is unknown; however, it is clearly sufficient to have caused the evolution of a number of phorid-specific defense behaviors.

## THE RECEIVING LOCATION

### DESCRIPTION OF FAUNA IN AREA OF PROPOSED AGENT INTRODUCTION

Evaluation of potential non-target impacts generally begins with identification of potential hosts that are closely related to the target host. Black and red imported fire ants have two native fire ant congeners in the southeastern United States: the tropical fire ant, *Solenopsis geminata* (Fabricius), and the southern fire ant, *Solenopsis xyloni* (MacCook). Both ants can be pests, but they rarely reach the same high densities of their imported cousins. The invasion of imported fire ants generally results in a dramatic decline of the number of *S. geminata* colonies and the elimination of all *S. xyloni* populations (Porter, 2000). Two native desert fire ants, *Solenopsis aurea* Wheeler and *Solenopsis amblychila* Wheeler, occur in the southwestern United States. These species are much less common, and it is generally assumed that they occur in habitats too dry for direct competition with imported fire ants. A dozen or so species of thief ants also occur in the southern United States; while these ants are also in the genus *Solenopsis*, they are not potential hosts of decapitating flies because of their extremely small size.

Other genera of ants in the subfamily myrmicinae are the next closest relatives of fire ants, followed by ants in other subfamilies. However, ant head size is probably more critical than subfamily, judging by the fact that species of *Pseudacteon* flies have been found parasitizing similar-sized ants in the subfamilies Formicinae, Dolichoderinae, and Ecitoninae (Disney, 1994). Very small ants (< 0.4 mm, head width) and large ants (>1.6 mm, head width) apparently lack *Pseudacteon* parasites.

In contrast to more conspicuous insects like butterflies, no ant species are listed as rare or endangered. A number of native ants have very interesting habits to myrmecologists, but only perhaps *Pogonomyrmex* harvester ants and *Atta* leaf-cutter ants would stand out as iconic species to the public where they occur. Nevertheless, ants in general play important roles in

food webs (Folgarait, 1998), and at least one threatened vertebrate, the Texas horned lizard (*Phrynosoma cornutum* (Harlan)), depends heavily on native ants, particularly harvester ants, for food (Blackshear and Richerson, 1999). It is therefore important that potential fire ant biological control agents be restricted as much as possible to imported fire ants.

Fire ants and a few other species of ants are considered beneficial to a few crops, especially sugarcane in which they help control the sugarcane borer (*Diatraea saccharalis* Fabricius). Consequently, some sugarcane growers vociferously object to biological control efforts directed at imported fire ants. However, the benefits to sugarcane production would be less clear if the comparison was between “fire ants and other ants” rather than “fire ants and no ants” (Adams *et al.*, 1981). In Texas rangelands, ticks are virtually absent where fire ants have invaded, so ranchers are likewise concerned about imported fire ant biological control (Fleetwood *et al.*, 1984). In regard to these special concerns, it is important to keep in mind that the net impacts of imported fire ants across all areas of agriculture, human health, and wildlife are overwhelmingly negative (Lard *et al.*, 2001; Wojcik *et al.*, 2001).

## THE TESTING PLAN: ANALYSIS OF METHODS

Four categories of pre-release host range studies have been conducted with fire ant decapitating flies: (1) literature searches, (2) general field observations, (3) field tests in the native range, and (4) laboratory tests in quarantine. In addition, (5) a battery of food preference tests were conducted to determine if the flies were attracted to food items that might make them a nuisance or a potential disease vector. Finally, (6) several post-release tests were conducted to test the effectiveness of pre-release predictions.

### LITERATURE SEARCHES

Literature searches provided important data about hosts in the field, geographic distribution, and the hosts of related species of phorids. The taxonomic publications of Thomas Borgmeier in Brazil were especially important (Borgmeier, 1921, 1925, 1962; Borgmeier and Prado, 1975).

### FIELD OBSERVATIONS

Information from the literature was also supplemented by additional field observations in the past decade as we and our colleagues conducted studies of the ecology and behavior of phorids and their hosts in South America

### FIELD TESTS IN THE NATIVE RANGE

Field tests in the native range were conducted with *Pseudacteon* flies to confirm data from the literature that indicated a general host specificity to fire ants. The first set of tests was conducted by setting out clusters of four to nine trays with different species of ants, one of which contained South American fire ants (Porter *et al.*, 1995c). The second set of tests was conducted by setting out tropical fire ants (*S. geminata*) in trays for 30 minutes followed by red fire ants for a similar amount of time (Porter, 1998b). These tests were particularly useful because *S. geminata* occurs in both Brazil and the United States.

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## LABORATORY TESTS IN QUARANTINE

The tropical fire ant (*S. geminata*) was selected as the primary native ant species to test because it was the most common native congener in the United States to imported fire ants. The theory was that a fly that would not attack a fire ant congener would also be very unlikely to attack ants in other genera. A second native fire ant, *S. xyloni*, was added for tests with *P. curvatus* after tests showed that *P. curvatus* would develop in *S. geminata*. Both *S. geminata* and *S. xyloni* were used in most subsequent tests in an attempt to be more thorough and because data collected so far indicate that *Pseudacteon* flies are more likely to parasitize *S. xyloni* than *S. geminata*. Native non-*Solenopsis* ants were also added if flies were capable of developing in either species of native fire ant. Non-native ants were selected from as many different genera as possible – giving preference to species of appropriate size for the fly being tested. Specificity tests have not been conducted with either of the desert fire ants (*S. aurea*, *S. amblychila*) because colonies of these species are difficult to obtain and their desert habitat would seem to make them less susceptible to *Pseudacteon* flies from South America. Furthermore, it was judged that negative impacts on the desert fire ants were unlikely to exceed those found on *S. geminata* and *S. xyloni* and that, even if they were somewhat higher, this would be unlikely to stop the release of a promising agent that would be far more likely to help protect whole communities of ants by its impacts on imported fire ants.

**No-choice tests** No-choice tests were the first tests conducted in the laboratory. The objective was to determine whether a particular species of fly had the motivation and capability of attacking a potential host ant when no other alternatives existed. No-choice tests were initially conducted with flies that had been hand carried up from South America and immediately tested in quarantine in the one to three days before they died (Porter, 1998a). Because the viability and motivational status of many field-caught flies were suspect, sequential tests were used. Gilbert and Morrison (1997) and Morrison and Gilbert (1999) tested individual flies with imported fire ants, then with native fire ants, and finally with imported fire ants again. Flies not showing activity against imported fire ants were not used in tests with native fire ants. This method tested the specificity of flies known to be motivated to attack the normal host, thus reducing the chances of false negatives due to stress or age. Porter and Alonso (1999) also used sequential testing. Half of their tests were done first with imported fire ants and then with native ants; the other half of the tests were done first with native ants and then with imported fire ants. This method assessed both the host specificity of flies that had been primed for attack with imported fire ants and those that had not.

Additional no-choice tests have also been conducted using flies reared from laboratory cultures in quarantine. For these tests, 15-20 flies were generally placed into attack boxes (Figure 3); several boxes usually contained test ants and the others contained imported fire ants used as controls or standards. Five to nine replicate trays were run for each species tested. The percent of hovering flies, attack rates, and parasitism rates were determined for each test box. Test flies remained in the boxes until they died of old age or died of the perils of attacking fire ants, usually 1-3 days. The advantage of using flies from laboratory cultures was that much larger numbers of flies could be tested over their full adult lifespan. The disadvantage was that 6-12 months of labor were already invested in rearing the flies before determining whether they



would be suitable for field release. This risk was acceptable after we determined from the previous tests that most flies would likely be suitably host specific.



Figure 3. Attack boxes used in no-choice tests at the USDA quarantine facility in Gainesville, Florida. The electric motor on top of the box drives a cam that raises and lowers the green and black lids inside the box every 15 minutes so that ants trail back and forth while the flies attack. Photos: S. D. Porter.

Another alternative to transporting short-lived flies to the United States for specificity tests would be to transport *geminata* complex ants (*S. geminata* and *S. xyloni*) to quarantine facilities in South America, where it would be much easier collect flies for testing. This could be done safely if sterile workers without queens or sex brood were used. Conducting host range tests in South America with potential North American hosts is a tempting possibility, but this would entail resolving serious issues of trust, responsibility, and perceptions before permits could be obtained. Host range studies involving the importation of pest ant biotypes back to their country of origin, however, has been arranged for *Pseudacteon* parasitoids of Argentine ants (Orr *et al.* 2001).

**Choice tests** Paired choice tests were usually only conducted after no-choice tests indicated that a particular species of fly was capable of attacking and developing in a native non-target fire ant host (Porter, 2000; Vazquez *et al.*, 2004). The objective was to determine whether the flies were likely to have strong preference for imported fire ants when they occurred in micro-sympatry (i.e., when near the same resource) with native fire ants. Choice tests were also used to assess host range preferences of flies attacking fire ants within the *saevissima* complex in South America. Choice tests were never done between *Solenopsis* and non-*Solenopsis* ants because the *Pseudacteon* test species rarely attempted to attack non-*Solenopsis* ants and never successfully parasitized them. Paired choice tests were conducted in specially modified attack chambers, each of which contained two parallel trays in the bottom – one for each of the host pairs. Tests were generally run for two to three hours with 10-15 flies in each tray. The paired chambers were also used to test two non-*Solenopsis* ants at a time because it was anticipated that flies would not be motivated to attack either ant species.

#### FEEDING PREFERENCE TESTS

Many kinds of flies can be a nuisance or even a health hazard if they are attracted to humans, animals, fruits, prepared foods, carrion, feces, or dung. While not directly related to host range, it was important to determine whether *Pseudacteon* flies were likely to vector diseases or be-

come a nuisance problem. To investigate this potential, unfed flies were placed into trays with a smorgasbord of potential food items (Porter, 2000). Chi-square tests were used to see if they were attracted to any of the potential food items more frequently than they were to moist tissue paper.

#### POST-RELEASE SPECIFICITY TESTS

Post-release specificity tests were conducted in the field in order to determine whether pre-release assessments of host specificity were accurate predictions of what happened in the field (Morrison and Porter, 2005; Vazquez and Porter, 2005). These tests were conducted in two ways. The first was to look for flies attracted to disturbed native and imported fire ant mounds. The second was to place trays of ants in the field, as was done in South America. In tests with trays, native fire ants or native ants from other genera were put out in trays for 20-30 minutes, after which they were replaced with trays of imported fire ants to confirm the presence of the flies. Then the imported fire ants were removed and the native ants were replaced to see if they would be attacked by motivated flies after they had been attracted to the trays.

## TEST RESULTS AND INTERPRETATIONS

#### LITERATURE SEARCHES

In the literature, ants were listed as the presumptive host for all known flies in the genus *Pseudacteon* (Wasmann, 1918; Borgmeier, 1921, 1925, 1969; Borgmeier and Prado, 1975; Disney, 1994). Furthermore, virtually all phylogenetically related phorid genera are also apparently ant parasites (Brown, 1993; Disney, 1994). The life cycle of *Pseudacteon* flies (Porter *et al.*, 1995a) strongly suggests a high degree of host specificity. In particular, the puparium is highly modified to fit snugly in the head capsule of a decapitated ant. This information suggests that only ants and probably only ants of a particular size range would be suitable hosts. Similarly, the well developed and distinctive ovipositors also suggest a high degree of host specificity.

The literature further indicated that almost all *Pseudacteon* species are only attracted to worker ants in a single genus. One species (*P. borgmeieri* Schmitz) was reported to attack both *Solenopsis* and *Camponotus* ants, but on investigation, this turned out to be a translational error (Porter *et al.*, 1995c). Another rare species (*Pseudacteon convexicauda* Borgmeieri) has been collected hovering over both *Solenopsis* and *Paratrechina* ants, but field observations suggest that it is actually a parasite of *Paratrechina* workers (Porter and Pesquero, 2001). Perhaps it is occasionally collected over fire ants when *Paratrechina* ants are mixed in. *Pseudacteon formicarum* (Verrall) in Europe has been reported hovering over several genera of ants, but Wasmann argued it was only a parasite of *Lasius* ants (Wasmann, 1918). Ultimately, rearing tests will be necessary in order to resolve questions about this species and the previous one.

Finally, the literature indicated that *saevissima* and *geminata* complex fire ants have distinct communities of *Pseudacteon* parasites. At least four species of flies are known to parasitize *S. geminata* and/or *S. xyloni* in the United States, but they were never collected attacking imported fire ants (both in the *saevissima* complex) in the United States, even though they clearly would have had the opportunity to do so (Smith, 1928; Morrison *et al.*, 1999b). Similarly,

*Pseudacteon* parasitoids of *saevissima* complex fire ants have not been reported attacking *geminata* complex fire ants even though there is broad geographic overlap between these two groups in northern South America. Two species of flies, *Pseudacteon solenopsisidis* (Schmitz) and *Pseudacteon wasmanni* (Schmitz), were reported in the literature as attacking fire ants in both the *geminata* complex and the *saevissima* complex (Disney, 1994), but the reports for *geminata* turned out to be an early misidentification (Schmitz, 1914) of what was almost surely *saevissima* complex ants (Fowler *et al.*, 1995b).

In the Americas, *Pseudacteon* phorids can be typified as fire ant specialists because most species are fire ant parasitoids (Disney, 1994). The observation that *Pseudacteon* flies have only colonized a few non-*Solenopsis* ant genera over evolutionary time, and the fact that North American *Pseudacteon* flies using *geminata* complex ants have failed to colonize either red or black imported fire ants in 7-8 decades of exposure is testimony to the powerful constraints against switching hosts. These constraints appear to relate to the use of host pheromones in locating workers to parasitize (Morrison and King, 2004). Moreover, the fact that host ants are under pressure to evolve unique chemical signals may account for the species-level specialization we often observe in this system.

#### FIELD OBSERVATIONS

With regard to field observations taken in the course of behavioral and ecological studies, the Gilbert and the Porter research groups and their various colleagues in Brazil and Argentina began intensive field observations in the early 1990s. The host specificity patterns apparent in the literature were confirmed by hundreds of hours of field observations taken over baits where *saevissima* complex ants interacted with many other ant genera ( $n > 20$ ). In summary, information in the literature, supplemented by extensive field observations, indicate that *Pseudacteon* flies that attack fire ants would be specific to fire ants. Furthermore, it is also likely that flies that attack imported fire ants would prefer imported fire ants over native fire ants.

#### FIELD TESTS IN THE NATIVE RANGE

The host specificity of *Pseudacteon* flies was initially tested in the field at three locations in South America with 23 species of ants from 13 genera (Porter *et al.*, 1995c). *Pseudacteon* flies, primarily *P. litoralis* and *P. wasmanni*, but also lower numbers of *P. tricuspidis*, *Pseudacteon pradei* Borgmeier, *P. curvatus*, and *P. borgmeieri*, were attracted only to *Solenopsis* fire ants. Three individuals of two species of flies (*P. wasmanni* - 2, *P. pradei* - 1), however, were attracted to a tray containing black *S. geminata* fire ants.

A second set of field tests was conducted with three colonies of black *S. geminata* and three colonies of *saevissima* complex ants. Trays with these colonies were set out two times at each of two sites near Rio Claro, Brazil (Porter, 1998b). When the *S. geminata* colonies were set out, they initially attracted no flies; however, when the *saevissima* complex ants were set out, flies were always attracted to each tray with *saevissima* ants (12 of 12 opportunities). When all the trays were placed together at one location, flies were again attracted to all of the *saevissima* trays, but only one *S. geminata* tray briefly had a *P. litoralis* fly that hovered but did not attempt to oviposit. When the *saevissima* trays were removed, leaving only *S. geminata* trays, a total of five flies hovered over a *S. geminata* tray on four of 12 occasions. One fly (*P. wasmanni*)

was observed systematically attacking *S. geminata* workers. When the *saevissima* trays were returned, all of the flies selected ants in the *saevissima* trays. At the end of the experiment, 588 fly larvae were reared from the three *saevissima* trays, compared to 12 larvae from the *S. geminata* trays. The 262 flies that emerged from the *saevissima* trays were 52% *P. tricuspis*, 39% *P. litoralis*, 5% *P. wasmanni*, *P. pradei* 3%, and 0.4% *P. curvatus*. No flies emerged from the *S. geminata* trays, but at least three of the pupae from these trays were *P. wasmanni*.

The results of these field tests in Brazil showed that the *Pseudacteon* species that attack fire ants were not attracted to ants in other genera. Tests with *S. geminata* showed that *P. litoralis* and *P. tricuspis* largely ignored *S. geminata* workers. However, *P. pradei* and *P. wasmanni* would hover over *S. geminata* workers and *P. wasmanni* was probably capable of completing development in *S. geminata*. Nevertheless, when both *saevissima* complex workers and *S. geminata* complex workers were present, all of the *Pseudacteon* flies at the test site selected *saevissima* complex workers.

#### LABORATORY HOST RANGE TESTS

**No-choice tests with native congeners** Laboratory no-choice tests in quarantine facilities show that *P. tricuspis* and *P. litoralis* have a high degree of host specificity for the red imported fire ant *S. invicta* over the native fire ants *S. geminata* and *S. xyloni* (Table 1). Females of *P. litoralis* and *P. tricuspis* occasionally hovered over native fire ant workers but only at 0-15% of the rates that they did over *S. invicta* workers. Oviposition attempts were even rarer. *Pseudacteon litoralis* females have never successfully parasitized *S. invicta* workers and *P. tricuspis* females have only succeeded once when dead *S. invicta* workers were mixed in with live *S. geminata* workers. *Pseudacteon wasmanni* flies also showed high specificity to the red imported fire ant when compared to *S. geminata*, but more tests will be necessary because test numbers were low (Table 1) and the field trials indicated the potential for this species to develop in native fire ants (Porter, 1998b). Preliminary tests also indicated that *Pseudacteon nudicornis* Borgmeier is highly host specific. The small unnamed species near *P. obtusus* from Campinas, Brazil, was also highly host specific to imported fire ants. *Pseudacteon obtusus* flies from Herradura and Corrientes, Argentina were able to attack small numbers of native fire ants and development was confirmed in the Herradura flies (Table 1). *Pseudacteon curvatus*, *P. borgmeieri*, and *Pseudacteon nocens* Borgmeier were the least host specific of the flies tested as far as their attack rates (Table 1). *Pseudacteon curvatus* females hovered over native fire ants at about 2/3 of the rate over the red imported fire ant, and parasitism rates ranged from 0-35% of the rate for *S. invicta* depending on the host and the origin of the fly. Several *P. borgmeieri* and *P. nocens* flies also readily attacked native *S. geminata* fire ants (Table 1), but no data are available about whether they are able to successfully parasitize them. While 36% of *P. nocens* females attacked *S. geminata*, they did so at 1/6th the rate with *S. invicta* (Gilbert et al., unpubl. data). *Pseudacteon cultellatus* Borgmeier attacked *S. geminata* in low numbers (Table 1), but sample sizes are still too low to be precise and it is not known whether they can parasitize native fire ants.

**Choice tests with native congeners** Paired choice tests were run with *P. curvatus* from Las Flores and Formosa (both locations in Argentina) and *P. obtusus* from Herradura, Argentina. These tests were undertaken because both species had the ability to attack and develop in native fire ants; therefore, it was important to know whether these flies had a preference for



Table 1. Percentage rates of attack or parasitism of native fire ants (*S. geminata*, *S. xyloni*) compared to rates for the target host the red imported fire ant (*S. invicta*) for ten species of South American *Pseudacteon* flies.

Fly Parasitoid Species Source (# tested)	Attack Behavior		Parasitized Workers		Literature Source
	<i>S. geminata</i> (% of rate on <i>S. invicta</i> )	<i>S. xyloni</i>	<i>S. geminata</i> (% of # on <i>S. invicta</i> )	<i>S. xyloni</i>	
<b><i>Pseudacteon litoralis</i></b>					
Campinas, BR (23)	9	—	0	—	Gilbert & Morrison, 1997 <sup>a</sup>
Jaguaríuna, BR (68, 51)	7	—	0	—	Porter & Alonso, 1999 <sup>b</sup>
San Justo, AR (20, 15)	0	3	0	0	Porter, unpublished <sup>c</sup>
<b><i>Pseudacteon tricuspis</i></b>					
Campinas, BR (25)	4	—	0	—	Gilbert & Morrison, 1997 <sup>a</sup>
Jaguaríuna, BR (84, 72)	5	—	0	—	Porter & Alonso, 1999 <sup>b</sup>
Formosa, AR (27, 27)	8	15	0	0	Porter, unpublished <sup>c</sup>
<b><i>Pseudacteon wasmanni</i></b>					
Campinas, BR (18)	11	—	0	—	Gilbert & Morrison, 1997
Jaguaríuna, BR (9)	0	—	0	—	Porter & Alonso, 1999 <sup>b</sup>
<b><i>Pseudacteon nudicornis</i></b>					
Santiago del Estero, AR (6)	0	—	—	—	Gilbert, <i>et al.</i> , unpublished <sup>a</sup>
<b><i>Pseudacteon</i> sp. near <i>obtusus</i></b>					
Campinas, BR (18)	0	—	—	—	Morrison & Gilbert, 1999 <sup>a</sup>
<b><i>Pseudacteon obtusus</i></b>					
Herradura, AR (102, 102)	14	29	4	13	Porter, unpublished <sup>c</sup>
Corrientes, AR (8)	13	—	—	—	Gilbert, <i>et al.</i> , unpublished <sup>a</sup>
<b><i>Pseudacteon curvatus</i></b>					
Campinas, BR	65	—	12	—	Gilbert & Morrison, 1997 <sup>a</sup>
Las Flores, AR (180, 140)	11	71	6	35	Porter, 2000 <sup>c</sup>
Formosa, AR (150, 130)	64	77	0	13	Vazquez <i>et al.</i> , 2004 <sup>c</sup>
<b><i>Pseudacteon borgmeieri</i></b>					
Jundiai, BR (3)	67	—	—	—	Morrison & Gilbert, 1999 <sup>a</sup>
Buenos Aires, AR (2)	100	—	—	—	Morrison & Gilbert, 1999 <sup>a</sup>
<b><i>Pseudacteon nocens</i></b>					
Santiago del Estero, AR (61)	36	—	—	—	Gilbert, <i>et al.</i> , unpublished <sup>a</sup>
<b><i>Pseudacteon cultellatus</i></b>					
Santiago del Estero, AR (12)	8	—	—	—	Gilbert, <i>et al.</i> unpublished <sup>a</sup>

<sup>a</sup> Attack behavior data are calculated from the percentage of females that also attacked *S. geminata* after they had attacked *S. invicta*.

<sup>b</sup> Attack behavior data are calculated from the total number of flies attacking *S. geminata* as a percent of the number that attacked *S. invicta*.

<sup>c</sup> Attack behavior data are calculated from the mean number of flies hovering in attack mode during the observation period in *S. geminata* or *S. xyloni* boxes as a percent of the mean number observed in the *S. invicta* boxes.

imported or native fire ants when they co-occurred. For *P. curvatus*, the results were that 75-85% of the female flies preferred the imported fire ant over either native fire ant (Porter, 2000; Vazquez *et al.*, 2004). Females reared on *S. xyloni* retained a strong preference for *S. invicta*, indicating that host preferences are genetically hardwired rather than facultatively determined by rearing history (Porter, 2000). About 95% of *P. obtusus* females chose to attack *S. invicta* over either native species (Porter, unpublished data).

**Host range tests with saevissima complex fire ants** In addition to studying host range to determine impacts on non-target organisms, it is also important to determine whether the biological control agents being studied can successfully attack the target hosts. Adequate host range breadth to attack all target species is important because both the red fire ant, *S. invicta*, and the black fire ant, *S. richteri*, occur in the United States. Field collection data in South America showed that most species of decapitating flies were broadly distributed across the ranges of several species of fire ants such that they must use several different species as hosts (Borgmeier and Prado, 1975; Porter and Pesquero, 2001; Folgarait *et al.*, 2004). Additional studies have demonstrated that flies from a specific location are usually capable of attacking and parasitizing several species of fire ants in the *saevissima* complex (Porter and Briano, 2000; Folgarait *et al.*, 2002a; Folgarait *et al.*, 2002b). Nevertheless, early tests with *P. tricuspis* from Argentina suggested that flies collected from black fire ants preferred black fire ants (Porter *et al.*, 1997b). Subsequent laboratory tests have confirmed that *P. tricuspis* flies from red fire ants prefer red fire ants and *P. tricuspis* flies from black fire ants prefer black fire ants, although flies were capable of parasitizing either host (Porter, unpubl. data). Laboratory tests with *P. curvatus* collected from red and black fire ants showed the same pattern (Porter and Briano, 2000, Vazquez and Porter, unpublished data).

**No-choice tests with ants in other genera** No-choice tests have been conducted in quarantine with five species of flies (Table 2). About 2% of *P. litoralis* females (1/51), 5% of the *P. tricuspis* females (3/61), and none of the *P. wasmanni* females (0/6) hovered over ants in another genus and appeared to attempt oviposition (Porter and Alonso, 1999). The *P. curvatus* flies hovered over the native ants at about 14% of the rate that they did over *S. invicta* workers. In most cases, a few oviposition attempts were also observed (Porter, 2000). None of the *P. obtusus* flies hovered over any of the non-*Solenopsis* ants in the quarantine tests (Porter, unpubl. data).

All ant species in which oviposition attempts were seen were maintained for 4-5 weeks and observed for signs of parasitism. Two species of native phorid parasites were found; however, none of the test ants in other genera were parasitized by any of the five species of South American decapitating flies being tested.

#### FEEDING PREFERENCE TESTS

A thorough review of the literature showed no reports of *Pseudacteon* flies being attracted to fruit, animals, prepared food, carrion, feces, or dung in South America. None of the decapitating flies were ever attracted to the authors, their colleagues, or their lunches during many hours of collecting activities in the field (Porter, unpubl. data; Porter, 2000). The flies *P. litoralis* and *P. tricuspis* were not attracted to prepared foods, sugar solutions, oils, fruits, feces, or flowers during rearing tests in Rio Claro, Brazil (Porter, unpubl. data). *Pseudacteon tricuspis* flies from Buenos Aires, Argentina were also not attracted to various types of foods presented during rearing tests although they were observed to lap up sugar and honey water if these were encountered (Porter *et al.*, 1997b). *Pseudacteon curvatus* flies were tested with more than 50 potential food items, including fruits, vegetables, raw meat, prepared foods, carrion, and dung. The flies showed no more attraction to any of the test items than they did moist tissue paper balls used as controls; in fact, 75% of the flies never visited any of the test items (Porter, 2000).

Table 2. Genera of non-*Solenopsis* ants used in no-choice tests with phorid decapitating flies in quarantine; none of these ants were successfully parasitized.

<i>Pseudacteon</i> Fly Species	Non- <i>Solenopsis</i> Ant Genera (number of species) Tested
<i>Pseudacteon litoralis</i> <sup>a</sup>	<i>Aphaenogaster</i> (2), <i>Camponotus</i> , <i>Crematogaster</i> , <i>Neivamyrmex</i> , <i>Pheidole</i>
<i>Pseudacteon tricuspis</i> <sup>a</sup>	<i>Aphaenogaster</i> (2), <i>Camponotus</i> , <i>Crematogaster</i> , <i>Forelius</i> , <i>Pheidole</i>
<i>Pseudacteon wasmanni</i> <sup>a</sup>	<i>Aphaenogaster</i> , <i>Crematogaster</i>
<i>Pseudacteon curvatus</i> <sup>b</sup>	<i>Aphaenogaster</i> , <i>Camponotus</i> , <i>Crematogaster</i> (3), <i>Dorymyrmex</i> (2), <i>Forelius</i> , <i>Lasius</i> , <i>Leptothorax</i> , <i>Linepithema</i> , <i>Pheidole</i> (5), <i>Pseudomyrmex</i> , <i>Tetramorium</i> , <i>Trachymyrmex</i>
<i>Pseudacteon obtusus</i> <sup>c</sup>	<i>Aphaenogaster</i> (2), <i>Camponotus</i> (2), <i>Crematogaster</i> , <i>Cyphomyrmex</i> , <i>Dorymyrmex</i> , <i>Formica</i> , <i>Odontomachus</i> , <i>Pheidole</i> (2), <i>Pseudomyrmex</i> , <i>Tetramorium</i> , <i>Trachymyrmex</i>

<sup>a</sup>Porter and Alonso, 1999; <sup>b</sup>Porter, 2000 (Las Flores biotype); <sup>c</sup>Porter, unpublished data

### POST-RELEASE FIELD TESTS

Specificity tests conducted in the field about three years after *P. tricuspis* had been released in north Florida showed no signs of non-target effects (Morrison and Porter, 2005). Flies were not attracted to mounds of the native fire ant *S. geminata*. They were also not attracted to trays with native fire ants or to trays with 14 other species of ants in 12 different genera. These results were congruent with what had been predicted by laboratory and field host specificity tests (Gilbert and Morrison, 1997; Porter, 1998a; Porter and Alonso, 1999).

No-choice post-release specificity tests were also run with the Formosa biotype of *P. curvatus* about 8 months after field release (Vazquez and Porter, 2005). A few flies were attracted to trays with *S. geminata* ants, but the rates of attraction were less than 5% of those observed with the red imported fire ant, and the few flies that came generally hovered for a few minutes without attacking and then left. These results were much better than predicted by laboratory tests in which the hovering rate over *S. geminata* was about 66% of the rate for *S. invicta* for no-choice tests and about 15% for choice tests (Vazquez *et al.*, 2004). The native fire ants were not checked for parasitism because only one possible attack was observed and the flies had never successfully parasitized *S. geminata* fire ants in the laboratory. No *P. curvatus* flies were attracted to any of the 15 other species of ants in 12 other genera that were offered in test trays.

Post-release specificity tests have also been done with the Las Flores biotype of *P. curvatus* in Alabama, where they are established on hybrid (red x black) fire ants from a field release about three years earlier (Graham *et al.*, 2003). Some females were attracted to trays with the native fire ant *S. geminata* and attacks were commonly observed; however, the rate of parasitism in *S. geminata* workers was about 3% of the rates with *S. invicta* or hybrid fire ants (Porter and Graham, unpubl. data). This is a little less than would have been predicted by the laboratory tests (Porter, 2000). Tests with ants in other genera still need to be done.

## RISK ASSESSMENTS

***Pseudacteon tricuspis* and *Pseudacteon litoralis*** The risk to native fire ants from the *P. tricuspis* and *P. litoralis* is very small. Field tests show that these species are not attracted to *S. geminata* fire ants (Porter, 1998b). Even under forced laboratory conditions, almost all female flies refused to attack *S. geminata* workers (Gilbert and Morrison, 1997; Porter and Alonso, 1999). However, *P. tricuspis* will attack *S. geminata* under unusual circumstances and did, in one instance, complete development in an *S. geminata* worker (Porter and Alonso, 1999). A small risk to *S. geminata* is acceptable for four reasons. First, this species, while being native to the United States and other parts of the Americas, is a pan-tropical pest (Trager, 1991). In fact, it would probably be ranked as one of the most important exotic ant pests in the world (Williams, 1994). Fortunately for us, its densities here have never approached those of the imported fire ants (Porter, 1992). Secondly, this ant and its sister species, *S. xyloni*, already have at least four species of *Pseudacteon* phorids that attack them in the United States but that do not attack the imported fire ants (Morrison *et al.*, 1997). Consequently, it seems very unlikely that imported *Pseudacteon* species that are not even attracted to *S. geminata* could switch to a new host and out-compete the phorid parasites that have already coevolved with it. Thirdly, the range of *saevissima* complex fire ants in South America overlaps broadly with that of *S. geminata* (Trager, 1991); thus, most of the phorid parasites of *saevissima* complex ants have probably already had millions of years to make the jump to *S. geminata*, but without success. Fourthly, *S. invicta* is slowly displacing *S. geminata* from most of its range in the United States (Porter, 1992; Wojcik, 1994): in other words, the clear and present danger that *S. invicta* poses to *S. geminata* is much greater than the small risk that introduced *Pseudacteon* flies would have. This final argument is also applicable to *S. xyloni* because the imported fire ant *S. invicta* has totally eradicated *S. xyloni* from almost all of its former range in the southeastern United States. Consequently, we can wait and permit *S. invicta* to continue eradicating *S. xyloni*, or we can take a small risk with importing several parasitic flies that may help reverse this trend—especially as *S. xyloni* is a pest species in its own right (Smith, 1965).

***Pseudacteon curvatus*** The laboratory and field host range information indicate that release of *P. curvatus* may pose a small risk to native fire ants (Porter, 2000). Release of this fly, however, is much more likely to benefit native fire ants because imported fire ants are their principal competitors, and these flies will almost certainly have much a greater effect on imported fire ants than on native fire ants. In short, risks to native fire ants need to be balanced against potential benefits to native ants in other genera and numerous other native organisms that are negatively affected by imported fire ants, including numerous rare and endangered species. Nevertheless, release of this species in regions such as Texas where native fire ants and their phorid faunas persist in some local areas behind the invasion front was delayed until we gained a better perspective on how *P. curvatus* might affect the system and could judge the prospects of developing comparable “small phorid” alternatives quickly (Gilbert and Patrock, 2002).

Much of the prior discussion concerning risk for *P. tricuspis* and *P. litoralis* also applies to *P. curvatus*, but several additional considerations are also important in regard to the field release of *P. curvatus*: (1) *P. curvatus* and other *Pseudacteon* species will, at best, stress imported fire ant



populations, thus reducing their ability to compete with native ants. Consequently, there is no chance that releasing *P. curvatus* will eradicate *S. invicta* or any of the native fire ants. (2) *P. curvatus* is among the smallest species of *Pseudacteon* flies that attack *S. invicta* (Morrison *et al.*, 1997), and it only attacks small and medium-small fire ant workers. This makes *P. curvatus* an excellent complement for *P. tricuspis*, which only attacks medium and medium-large fire ants (Morrison *et al.*, 1999 a,b). (3) Native fire ants were never as abundant as the imported species currently are (Porter *et al.*, 1988; Porter, 1992; Vinson, 1994), so there is little or no likelihood that native fire ant would simply replace imported fire ants as community-dominating pests. (4) Finally, the Las Flores biotype was originally collected from black fire ants (*S. richteri*) and then reared for several years on red fire ants (*S. invicta*) in quarantine. Even after this time, the Las Flores biotype still preferred black fire ants in choice tests (Porter and Briano, 2000). Field releases of this biotype have resulted in establishment several times on black and hybrid imported fire ants in Mississippi and Alabama (Graham *et al.*, 2003; Vogt and Streett, 2003), but failed seven of seven times on red imported fire ants in Florida (Graham *et al.*, 2003). In contrast, the Formosa biotype of *P. curvatus* was originally collected from red fire ants in northern Argentina. Field releases of this biotype have succeeded four of four times on red imported fire ants in Florida and South Carolina (Vazquez and Porter, unpubl. data; Davis, Pereira and Horton, unpubl. data). These results contradict data from no-choice tests with the Las Flores biotype, which predicted that *P. curvatus* would do as well on black as red imported fire ants (Porter and Briano, 2000). Perhaps the choice tests better indicated whether the flies were able to detect potential hosts at the range of several meters or more, a skill probably not tested in our small no-choice test boxes. Whatever the reason, it appears that the Las Flores biotype from black fire ants was too host specific to succeed in the field on red fire ants. If so, then it almost certainly poses little threat of becoming established on our native fire ants as predicted in spite of the fact that it successfully attacked and developed in native fire ants in laboratory tests (Porter, 2000).

***Pseudacteon obtusus*** We now know, based on AFLP analysis (Kronforst, Gilbert and Folgarait, unpubl.) that the smaller form of *P. obtusus* (Porter and Pesquero, 2001) is, in fact, a separate species. So far, this smaller species has shown no inclination to attack anything except *saevissima* complex ants. The risk of releasing the large form of *P. obtusus* from Herradura and Corrientes, Argentina, is intermediate between releasing *P. curvatus* and the two larger species *P. tricuspis* and *P. litoralis*. *Pseudacteon obtusus* attacked native fire ants, although at greatly reduced rates. However, an extremely high preference for imported fire ants in host choice tests (over 95%; Porter, unpubl. data) suggests that *P. obtusus* may be very specific to imported fire ants under field conditions. *Pseudacteon obtusus* showed no inclination to attack native ants in other genera.

**Other *Pseudacteon* species** Data for the remaining *Pseudacteon* species in Table 1 are mixed, but it is likely that further tests will show that all are at least as host specific as *P. curvatus*. The initial results from *P. nudicornis* and the small species near *P. obtusus* indicate that both species will be highly host specific. The five *P. borgmeieri* flies tested indicate low host specificity (Table 1); however, the failure of this species to successfully parasitize two South American fire ants in the *saevissima* complex (Folgarait *et al.*, 2002b) suggests that it is likely to fail to successfully parasitize the native fire ants in the United States. If *P. wasmanni* (Porter, 1998b) or any of

the other species can complete development in native fire ants, then it will be necessary to test them against ants in other genera. A complete set of tests have not been run with *P. wasmanni*, *P. borgmeieri*, *P. nocens*, *P. cultellatus*, *P. nudicornis*, or the new species near *P. obtusus* because we have not yet been able to rear these species in the laboratory. The low test numbers in Table 1 are due to difficulty in to collecting and transporting adult flies to the United States before they die. As discussed previously, this problem could be partially solved by conducting the tests in South American quarantine facilities, but this would likely require considerable effort and justification to make the necessary arrangements.

#### GENERAL RISK ASSESSMENT SUMMARY FOR FIRE ANT DECAPITATING FLIES

Field introductions of South American fire ant decapitating flies in the United States began after careful analyses of risks and benefits as elaborated in three Environmental Assessments for field release, which the authors separately prepared with and for officials at USDA/APHIS five, seven, and nine years ago. Below are the conclusions of the risk assessments submitted in support of requests for field-release permits.

1. ***Fire ant decapitating flies will not be a risk to plants, crops, or any agricultural products and may provide many benefits.*** Adult flies are not attracted to fruits or vegetables (Porter, 2000). Immature flies do not develop in or on plants. These flies may also substantially benefit agriculture by reducing fire ant damage in citrus, soybeans, potatoes, corn, sorghum, hay, etc. (Lofgren, 1986).
2. ***Fire ant decapitating flies pose no health risk for humans and may provide considerable health benefits.*** Adult flies are not attracted to humans, human wastes, or human food products. Immature flies pose no threat of developing in human tissues because of their specialized life history. However, if these flies prove to be successful biological control agents, they would be of considerable medical benefit to several hundred thousand people who are severely allergic to even a single fire ant sting (~1% of the population; Vinson, 1997) and to tens of thousands of small children every year who are stung repeatedly by hundreds of fire ants when they accidentally step into the mounds.
3. ***Fire ant decapitating flies pose no health risk to livestock or other domesticated animals and may provide health benefits as well.*** As noted above, these flies are not attracted to vertebrates, nor can they develop in vertebrate tissues (see above). Furthermore they are not attracted to, nor can they develop in, animal excrement. These flies, however, may provide health benefits to livestock by reducing the incidence of fire ant stings, especially to newly born animals.
4. ***The introduction of these flies will not be a risk to native wildlife or any native arthropods except perhaps some ants. Furthermore, they may considerably benefit natural biodiversity.*** Highly specialized ovipositors, oviposition behavior, host preferences, and pupation habits preclude conceivable risks to any organisms except ants. If these flies are able to help reduce fire ant populations, they would considerably benefit natural biodiversity (Porter and Savignano, 1990) and probably the survival of a number of rare or endangered vertebrates.

5. ***Pseudacteon species do not present a realistic risk to non-Solenopsis fire ants.*** None of the flies tested to date were attracted to other genera of ants in the field, and the few attacks that occurred in the laboratory did not produce any parasitized workers. It is theoretically possible for *Pseudacteon* phorids to switch to ant hosts in different genera because several species have done just that during the process of evolution (Disney, 1994). However, this is only likely to occur in evolutionary time scales of hundreds of thousands of years. Even then, such switches would be limited to a small subset of ants of similar size (Porter, 1998a). There is no conceivable possibility that a fire ant decapitating fly from South America would ever become a generalist parasite of ants.
  
6. ***Several of the Pseudacteon species proposed for release present a real but acceptable risk to Solenopsis geminata, Solenopsis xyloni and other native fire ants.*** The primary risk suggested by our specificity testing is that occasional attacks on non-target native fire ant species might occur. Several *Pseudacteon* species can also develop in *S. geminata* and *S. xyloni* under laboratory conditions (Table 1). However, all of these species are much more successful attacking imported fire ants than either of the native fire ant species tested. Furthermore, they also have a strong preference for imported fire ants over native fire ants when allowed to choose. These data justify a conclusion that *Pseudacteon* flies present a much greater risk to imported fire ants than to either of the native fire ants tested. This being the case, the likelihood is that these flies will actually benefit these native fire ant species a rather than harm them because imported fire ants are the primary enemy of native fire ants. Furthermore, risks to native fire ants need to be balanced against the possible benefits of this fly to hundreds of native arthropods and dozens of native vertebrates that appear to be threatened by high densities of imported fire ants. In short, a slight risk from secondary attacks pales in contrast to the benefit of finding an economic, self-sustaining, and target-specific biological control of imported fire ants.

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