PARASITOID CASE HISTORY: AN EVALUATION OF METHODS USED TO ASSESS HOST RANGES OF FIRE ANT DECAPITATING FLIES

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ABSTRACT

The first three papers in this section have discussed factors that affect the efficiency and success of laboratory host range tests. This paper presents an evaluation of how well those factors applied to our investigations of host ranges of fire ant decapitating flies in the genus *Pseudacteon* (Diptera: Phoridae). We initially discuss the nature of the fire ant problem (Hymenoptera: Formicidae: *Solenopsis* spp.) and the need for effective self-sustaining biological control agents. We briefly review the biology of *Pseudacteon* decapitating flies, the overall results of our host range tests, and the current status of field releases of these biological control agents. We conclude by discussing how well the recommendations of the three initial papers about 1) statistical procedures, 2) biotypes and cryptic species, and 3) experimental design, plus a recent book on the subject of host range testing, apply to our experiences with fire ant decapitating flies.

BACKGROUND OF PARASITOID SYSTEM

THE FIRE ANT PROBLEM AND NEED FOR SELF-SUSTAINING BIOLOGICAL CONTROL

The major problem with invasive fire ants (Hymenoptera: Formicidae: *Solenopsis* spp.) is that there are so many of them. In north Florida pastures, fire ant densities average 1,800-3,500 ants per square meter or about 1.5-3.0 metric tons of fire ants per square kilometer (Macom and Porter 1996; converted from dry weight to wet weight). Economic damage to agriculture, electrical equipment, and human health 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.

Fire ant populations in their South American homeland are about 1/5 as dense as populations normally found in North America (Porter *et al.* 1997). 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.* 1997). Escape from numerous natural enemies left behind in South America is the most apparent explanation for the intercontinental population differences. Classical or self-sustaining biological control agents are currently the only potential means for achieving permanent regional control of fire ants.

BIOLOGY OF *PSEUDACTEON* DECAPITATING FLIES

Information on the life history, phenology, and biogeography of South American *Pseudacteon species*, is accumulating (Porter 1998a; Folgarait, *et al.* 2002; 2003; 2005a; 2005b; Calcaterra *et al.* 2005). At least 20 species of *Pseudacteon* flies (Diptera: Phoridae) have been found attacking fire ants in South America (Porter & Pesquero 2001; Brown *et al.* 2003). Up to nine species of these flies have been found at a single site (Calcaterra *et al.* 2005). 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. Female flies usually contain a hundred or more eggs (Zacaro & Porter 2003). During oviposition, one egg is rapidly injected into the ant thorax with a short hypodermic shaped ovipositor (Fig 1A). 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 (Fig. 1B).

Figure 1. A) Female decapitating fly (*Pseudacteon*) preparing to inject an egg into the thorax of a fire ant worker (*Solenopsis*). B) Decapitated fire ant worker with a fly maggot consuming the contents of its head. UGA1390062, UGA1390063

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. Pupal development requires two to 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 at 20 °C, adult *Pseudacteon* flies may live up to two weeks (Chen *et al.* 2005); however, higher temperatures and activity associated with oviposition will shorten their lives to one to three days (Porter 1998a). Once phorid attacks commence, fire ant workers become keenly aware of the presence of the 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). Some fly species inhibit fire ant foraging as long as they are present, often for periods of several hours (Folgarait & Gilbert 1999; Wuellner *et al.* 2002). 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 & Gilbert 2002). 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 (Porter 1998a).

HOST SPECIFICITY OF *PSEUDACTEON* DECAPITATING FLIES

Based on the highly specialized behavior and life history of *Pseudacteon* flies, we conclude that they pose no threat to any arthropod except for ants (Porter 1998a). Based on the results of our host range tests (Porter & Gilbert 2004), we conclude that *Pseudacteon* decapitating flies are only a realistic threat to fire ants in the genus *Solenopsis*. None of the flies tested, to date, were attracted to other genera of ants in the field (Porter *et al.* 1995c, Morrison & Porter 2005c, Vazquez & Porter 2005) and the few attacks that occurred in the laboratory did not produce any parasitized workers (Porter & Gilbert 2004). 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). A major constraint on the evolution of host shifts and the broadening of host range is that phorids apparently use species-specific alarm pheromones to locate ant hosts (Vander Meer & Porter 2002). In almost eight decades of exposure to an expanding population of *S. invicta,* none of several species of *Pseudacteon* flies which attack native fire ants in North America have made the shift to the more abundant introduced species. All comparative and experimental evidence weighs heavily against the possibility that any of the fire ant decapitating flies from South America would ever become a generalist parasite of ants within ecological or microevolutionary timeframes.

Several of the *Pseudacteon* species proposed for release present a finite but acceptable risk to the native fire ants *Solenopsis geminata* (Forel) and *Solenopsis xyloni* MacCook (Porter & Gilbert 2004). The primary risk suggested by our specificity testing is that occasional attacks on these non-target native ants might occur. Several *Pseudacteon* species can also complete development in native fire ants. However, all of these species are much more successful at attacking imported fire ants than either of the native fire ant species tested. 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 either of the native fire ants tested. This being the case, the likelihood is that these flies will actually benefit native fire ant species rather than harm them because imported fire ants are the primary enemy of native fire ants (Porter 2000). Furthermore, risks to native fire ants must be balanced against the possible benefits of these flies to hundreds of native arthropods and dozens of native vertebrates threatened by high densities of imported fire ants (Wojcik *et al.* 2001). This small risk is justified, in light of the benefit of finding an economic, self-sustaining, and target-specific biological control of imported fire ants.

RELEASE AND ESTABLISHMENT OF DECAPITATING FLIES IN THE UNITED STATES

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 six, eight, and ten years ago. Three species of South American decapitating flies have been released in the United States. The first species was *Pseudacteon tricuspis* Borgmeier in Texas (Gilbert & Patrock 2002) and Florida (Porter *et al.* 1999). This fly attacks medium to medium-large fire ants and is especially abundant in the fall. A biotype of this species from near Campinas, Brazil is well established in eight states in the southeastern United States. Flies released in Florida have spread at least 180 km from their release sites (Porter *et al.* 2004). 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, Mississippi, and Tennessee (Graham *et al.* 2003; Vogt & Streett 2003; Parkman *et al.* 2005) and the other on red fire ants in Florida (Vazquez *et al.* 2005), South Carolina (Davis & Horton 2005), and Texas (L.G. unpublished). 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 (Summer 2003, Fall 2004). 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.

Studies of the impacts of these flies are ongoing, but field studies show that the impacts of a single species of fly (*P. tricuspis*) are not enough to rise above the 10-30% sensitivity of field tests (Morrison & Porter 2005a; 2005b). The introduction of additional species of decapitating flies and other natural enemies will increase the likelihood of permanently reducing imported fire ant populations in the United States.

EVALUATION OF RECOMMENDATIONS

The preceding authors in this section (Hoffmeister 2005; Hopper *et al.* 2005; Withers & Mansfield 2005) and those in a recent book (Van Driesche & Reardon 2004) have made a number of recommendations about procedures for assessing the host ranges of potential self-sustaining biological control agents from foreign countries. For the purposes of discussion,

we will divide these recommendations into six categories: 1) existing knowledge about the taxonomy and host specificity of potential biological control agents; 2) the importance of biotypes and cryptic species in host range tests; 3) selecting appropriate non-target organisms for testing; and 4) choosing the best ways to handle and select biological control agents for specificity tests; 5) experimental design for assessing host specificity; and 6) recommendations for proper statistical analysis of experimental data. We will proceed to discuss how well recommendations in each of these categories applied to our studies of the host ranges of fire ant decapitating flies.

EXISTING KNOWLEDGE

Explore literature. Generally, the first recommendation in assessing host ranges is to explore existing literature about identification and host records of potential biological control agents (Sands & Van Driesche 2004; Hoddle 2004). This is important advice. When we searched the literature, we found that all *Pseudacteon* species with host records had been collected attacking ants. We also found that more than 20 species of *Pseudacteon* flies had been described that attacked *Solenopsis* fire ants (Borgmeier 1925; 1962; 1969; Borgmeier & Prado 1975; Disney 1994). Indeed it appeared that *Pseudacteon* had diversified in a fire ant adaptive zone.

Contact experts. Hoddle (2004) recommended that taxonomists, museum curators, and other experts should be contacted for information. Contacting experts provided us with a wealth of information early in our programs. In particular, phorid specialist, Brian Brown shared his "*Pseudacteon* scrapbook" with us. This resource included references, descriptions, and illustrations for most of the species of flies that attacked fire ants. He also assisted with identifications when existing keys to the genus proved marginal and he provided taxonomic advise on numerous other occasions. David Williams and Don Feener provided additional literature about *Pseudacteon* flies as well as advise about their biology. Harold Fowler introduced SDP to these flies in the field. Roberto Brandão provided access to Thomas Borgmeier's collections at the Museum of Natural history in São Paulo. Roger Williams and Angelo Prado were also consulted about work they had done with these flies. In short, our colleagues provided an important foundation on which we were able to build.

Identification errors. Sands & Van Dreische (2004) warn that care must be taken to evaluate and validate old host records because some are not reliable. Indeed, we found two instances where improper identification of ant host records made it appear that three species of flies were less specific than they really are (Porter & Gilbert 2004). We also found evidence that a fourth species is likely more specific than generally reported (Porter & Gilbert 2004).

BIOTYPES AND CRYPTIC SPECIES

Hopper *et al.* (2005) caution that host range testing needs to be done on each new population of biological control agents being considered for field release. This is because cryptic species or biotypes can have different degrees of host specificity. We found this to be true with at least two species of *Pseudacteon* flies. In particular, we found that *P. tricuspis* appears to be two cryptic species, one of which attacks red fire ants and the other of which attacks black fire ants (Porter and Pesquero 2001). Similarly, we found that a biotype of *P. curvatus* collected from black fire ants in Buenos Aires, Argentina could not be established on red fire ants in the

United States while a biotype of *P. curvatus* originally from red fire ants in Formosa, Argentina was easily established on red fire ants in the United States (Vazquez *et al.* 2005). We also found that the two *P. curvatus* biotypes differed in their abilities to attack and develop in the two non-target native fire ants in North America (Porter 2000; Vazquez *et al.* 2004). These data indicate that each new population of a biological control agent needs to be screened for host specificity before field release, at least until the variability of host specificity is well understood within a particular species or genus. However, we do not think it appropriate to require separate permits for each new biotype of a species unless the new introduction falls outside of the host-specificity envelope already permitted for that species.

SELECTING NON-TARGETS FOR TESTING

Barratt *et al.* (1999) recommend that host range tests begin with closely related species in order to maximize the probability of identifying potential non-target host species. If closely related hosts are not suitable hosts, then additional testing with more distantly related organisms can often be greatly reduced because of the low probability that they would be suitable hosts. We generally agree with this line of reasoning. However, we initially tested more distantly related ant hosts to confirm literature observations that these flies were likely limited to ants in the genus *Solenopsis* (Porter *et al.* 1995c). If this screening test had shown broader than expected host ranges, further work with some or all of the fly species may have been abandoned. However, once we were convinced that *Pseudacteon* flies were likely very host specific, we focused our host range tests on the near native congener *S. geminata* and later on another native congener *S. xyloni* (Porter & Gilbert 2004). Two species of flies (*P. tricuspis* and *P. litoralis*) were not able to attack and develop in the native fire ants. Therefore, they were only tested with an abbreviated number of ants from other genera (Porter & Gilbert 2004). However, two species of flies (*P. curvatus* and *P. obtusus*) were capable of developing in one or more of the native congeners (Porter & Gilbert 2004) and as a result, they were both tested with a full battery of appropriately sized native ants from other genera (Porter 2000; Porter & Gilbert 2004).

HANDLING AND SELECTING BIOLOGICAL CONTROL AGENTS FOR TESTS

Withers & Browne (2004) and Withers & Mansfield (2005) make a number of suggestions for handling and selecting biological control agents for host range tests. Their suggestions are designed to "maximize the probability of attack on non-target species" in laboratory tests. Basically, their suggestions were to: 1) test biological control agents in groups, 2) use both naïve and experienced females, 3) select large females over small ones, 4) rear test agents on alternate hosts when possible, 5) deprive females of food prior to the test to increase motivation to oviposit, 6) use females deprived of oviposition opportunities for an appropriate amount of time, 7) test pro-ovigenic agents when young, and 8) use small test chambers. Several additional suggestions related to plant substrates, diet, and mating were generally not applicable to *Pseudacteon* flies.

1. Test in groups. This is a good recommendation for *Pseudacteon* flies. We have tested flies individually (Gilbert & Morrison 1997) but our preference is to test groups of 6-15 females when availability permits (Porter 2000; Folgarait *et al.* 2002; Vazquez *et al.* 2004; Porter & Gilbert 2004). A major benefit of groups is that a hundred or more flies can

easily be evaluated with only 8-12 test runs whereas individual testing would require a hundred or more test runs. Furthermore, tests with individual flies are often not dependable for many reasons including mating failures, ants killing flies, sick flies, no motivation to oviposit, etc. Finally, group testing is biologically normal because most *Pseudacteon* species attack gregariously in the field.

2. Naïve and experienced females. We used naïve females when using lab-reared flies and experienced females when using field-collected flies. We did not find evidence that prior experience in the field restricted subsequent host acceptability in lab trials. To the contrary, we actually have some evidence suggesting that flies attacking *S. invicta* in the lab are primed to approach non-target ants if exposed to them while they are still motivated. Specifically, tests with two species gave slightly higher rates of oviposition attempts (albeit unsuccessful) on non-target ants after having recently attacked the target species (Porter & Alonso 1999). Similarly, motivation to attack was generally short lived after Gilbert & Morrison (1997) transferred flies from target to non-target ants.

3. Large females. Withers & Browne (2004) recommended the use of large females on the assumption that they would have more eggs to lay and consequently be more motivated to oviposit. The relevance of this recommendation depends on details of an insect's life history. In the case of *Pseudacteon* females it is probably better to use a mixture of all sizes. This is because fire ant workers vary greatly in size and large and small female phorids attack different sizes of host workers (Porter 1998). Furthermore, small females could be more motivated to lay eggs because, under some circumstances, they do not live as long as large flies (Chen *et al.* 2005), thus canceling any benefits of small versus large.

4. Rear on alternate hosts. The suggestion about testing the host range of agents reared on alternate hosts has merit in some systems, but is largely impractical for most *Pseudacteon* species because their production rate is either very low or non-existent on alternate hosts. We know of no instance in which a *Pseudacteon* species from South American fire ants could be successfully cultured on North American fire ants or vise versa. Nevertheless, we were able run a small test to see if *P. curvatus* flies reared on the native fire ant *S. geminata* switched from their normal preference to *S. geminata*. We found that flies reared on the alternate host (*S. geminata*) showed little or no inclination to attack the alternate host indicating that host preferences in this fly were more genetic than facultative (Porter 2000).

5. Deprive food. This recommendation has little relevance for phorid flies that attack fire ants. Although we routinely deprived *Pseudacteon* flies of food in our tests, this is because they show little interest in feeding and the presence of food in oviposition chambers appears not to have much effect on fly health or parasitism rates. Also, most *Pseudacteon* species appear to be pro-ovigenic (Zacaro & Porter 2003) so feeding does not facilitate egg development.

6. Deprive oviposition opportunities. This recommendation applies best to insects with longer life spans. Depriving phorid flies of oviposition opportunities to improve motivation in host range tests is probably not necessary and could be counterproductive.

Indeed, if anything, *Pseudacteon* females are more likely to approach novel hosts immediately after exposure to normal host ants. *Pseudacteon* flies are usually very short lived when ants are available to attack (1-4 days) and oviposit most vigorously when they are young.

7. Test pro-ovigenic agents when young. Withers & Browne (2004) stated that pro-ovigenic agents would likely be best tested when they were young because they are often short-lived while synovigenic agents needed to be tested after eggs have matured and are ready to be laid. This is good advice for *Pseudacteon* flies because they are both pro-ovigenic and short lived. Nevertheless, we prefer tests which run for the full adult life of the flies because it gives them full opportunity to oviposit across all age ranges.

8. Small test chambers. We used small test chambers (Porter & Gilbert 2004) mostly because of limited space in our quarantine facilities; nevertheless, the use of small chambers in our tests rather than large ones probably did improve the likelihood of oviposition because the females could simply use visual or other short-range cues to find their hosts. This was good because it maximized the probability that test flies would oviposit in both target and non-target hosts. The down side of the small chambers is that we were not able to evaluate host specificity associated with long-range host detection.

EXPERIMENTAL DESIGNS

Van Driesche & Murray (2004) discuss the strengths and weaknesses of a number of experimental designs that have been used with host range testing including no-choice tests, choice tests, sequential tests, open field tests, preference ranking tests, and post-release tests. Withers & Mansfield (2005) evaluate choice and no-choice tests and recommend the use of either no-choice tests or a combination of no-choice and choice tests. During the course of our host range studies, we have used almost all of the experimental designs just mentioned.

No-choice tests. As recommended, we agree that no-choice tests are the best design for determining host ranges of *Pseudacteon* flies in the laboratory, at least when test flies are available in sufficient numbers either from the field or from a laboratory colony. No-choice tests were run with groups of flies (Porter 2000; Vazquez *et al.* 2004; Folgarait *et al.* 2002) for the entire life of the test flies. This allowed us to measure attraction rates, oviposition rates and most importantly parasitization rates.

Choice tests. We conducted binary choice tests when female flies in no-choice tests had demonstrated some abilities to attack and develop in non-target native fire ants (Porter 2000; Porter & Gilbert 2004). The objective was to determine whether females had a preference for the target species over the non-target native species. Our results showed strong preferences for imported fire ants over native fire ants. This preference data together with poor rates of development on native fire ants strengthened the argument that release of these flies would most likely benefit the native ants because of their impacts on imported fire ants (see specificity discussion under Background section).

We also used binary choice tests to screen ants in non-*Solenopsis* genera (Porter & Alonso 1999; Porter 2000; Porter & Gilbert 2004). However, these tests functioned like no-

choice tests since test flies always showed little or no attraction to ants from other genera and no test flies were ever reared from ants in other genera. Testing 3-4 species of non-target ants simultaneously would have increased testing efficiency. The drawback is that if flies had been attracted to any of the species of ants, we would have needed to repeat the tests to make sure that attraction to one ant species was not masking attraction to another (Withers & Mansfield 2005).

Sequential no-choice tests. Sequential no-choice tests were used to investigate the host specificity of several groups of flies transported into U.S. quarantine facilities from South America. Because of the short lifespan of field collected flies (2-5 days) and the time and expense required to hand carry these flies up from South America (1-2 days) we had very few flies and a very short time to conduct as many tests as possible. Gilbert & Morrison (1997) and Morrison & Gilbert (1999) chose to use an A-B-A pattern where the motivation of individual flies was tested against target ants (A) for five minutes and then against non-target ants (B) for 20 min, and finally against target ants (A) again to reconfirm motivation. In these tests, attacking flies moved from trays of target *S. invicta* (A*)* to trays of non-target *S. geminata* (B) initially approached, and sometimes attempted to oviposit in *S. geminata* workers. Typically however, motivation to attack carrying over from exposure to *S. invicta* was short lived and waned quickly after exposure to *S. geminata*. Porter & Alonso (1999) chose to test small groups of three flies in an A-B and a B-A pattern where some flies were first exposed to the target host while others were exposed first to the non-target host (each for periods of 60-90 minutes). This pattern controlled for any effects of recent exposure to the target host.

These sequential tests had two weaknesses: first all of the flies had been collected after they had prior experience with the target host and secondly test times (20 min. or 60-90 min.) could have been too short to overcome the effects of prior experience. Nevertheless, these limitations were largely unavoidable because of transport times, short life spans, and the fact that, at the time, the flies could not be cultured in the laboratory. Fortunately, results from these tests were equivalent to larger no-choice tests run later indicating that prior experience as wild flies is not a major factor affecting host range tests with *Pseudacteon*.

Withers & Mansfield (2005) recommend that Gilbert & Morrison (1997) could have used an A-A-A pattern to control for time dependant effects and similarly that Porter & Alonso (1999) could have used an A-A pattern. We agree that this suggestion could have provided some useful information. However, since the numbers of flies were very limited and many of them only survived one test cycle, we do not feel that the value of this information would have justified using 1/3 of the available flies. In the case of Gilbert & Morrison (1997), the second exposure to the target host in the A-B-A cycle provided most of the information that would have been provided by an A-A-A cycle. In our opinion, activity in an A-A-A cycle would not have been directly comparable to activity in an A-B-A cycle because the presence of the target host caused greatly increased activity that generally sapped the vigor and longevity of test flies. Our challenge was to keep flies alive and vigorous through even a short A-B-A cycle. In the case of Porter & Alonso (1999), an A-A test would have proved that flies exposed first to the target host (A) retained sufficient vigor to attack the non-target host (B). However, in keeping with the behavioral observations noted above for the A-B-A tests, the data showed that test flies were actually slightly more likely to attack the non-target

ant after being exposed to the target ant than vise versa (3/36 versus 0/79 attacking flies, P=0.029, Fisher's exact test, data for two species of flies combined). Thus, for *Pseudacteon*, we consider the sequential no-choice test to be conservative in that it tends to over-estimate the tendency of these flies to attack non-targets.

Open field pre-release and post-release tests. We conducted several pre-release and post-release open field tests with *Pseudacteon* flies. The major advantage of open field tests is that they take into account the long-range search and discovery abilities of test organisms. The major disadvantages of open-field tests are that the selection of potential hosts in pre-release tests are limited to what is available in the country of origin while in post-release tests, the biological control agent has already been released and can rarely be recalled. For the first open field test Porter *et al.* (1995c) used an $AB_1B_2B_3B_n$ design where target ants (A) were presented simultaneously with a menu of non-target ants (B). In subsequent papers (Porter 1998b; Morrison & Porter 2005c; Vazquez & Porter 2005), authors used a sequential B-A-B design where non-target ants (B) were presented for 30 minutes followed by target ants (A) and finally by non-target ants again (B). The advantage of this sequential design is that it allowed us to first determine if flies were attracted to non-target ants when no target ants were present and then it allowed us to determine if the flies would attack non-target ants after large numbers of flies had been attracted to the immediate area by the target ants. Van Driesche & Murray (2004) call post-release tests a "necessary step" in evaluating the accuracy of pre-release predictions. Results from our post-release tests confirmed that our pre-release predictions of host specificity were accurate for both species of flies that are currently established in the United States (Morrison & Porter 2005c; Vazquez & Porter 2005).

Statistical analyses. Hoffmeister (2005) discusses a number of important aspects of statistical design that apply to host range testing including proper controls, randomization, and pseudoreplication. He also discusses the potential importance of using power analyses to describe the power of statistical procedures to resolve differences between effects of interest.

Controls. Proper controls are vital to most kinds of statistical tests, but they are especially important to simple no-choice tests because the failure of a parasitoid to attack a potential non-target host could be due to poor test conditions or unhealthy parasitoids. To control for these possibilities, we randomly assigned test flies to simultaneous controls and treatments. On several occasions, we had to discard a run because the controls failed due to improper handling of the flies. Zilahi-Balogh *et al.* (2005) mention that the use of negative controls (tests without both a parasite and a host together) could have helped with interpretation of their oviposition tests. We did not use negative controls in any of our tests. Negative controls using ants that were not exposed to flies might have been useful in identifying ant mortality caused by parasitism prior to pupation of the parasite. However, based on random dissections of dead workers, we felt that pre-pupation mortality of host ants was not sufficiently large to justify the extra effort needed to quantify it.

Pseudoreplication and randomization. We attempted to avoid pseudoreplication in our tests by randomly assigning subjects to treatments and using experimental units that were independent of one another. However, in practice, flies were usually assigned to test groups using "haphazard randomization" and the locations of test trays were usually rotated sequentially among test groups so that whatever effect tray location might have would be uniformly

distributed across treatments. Finally, our host range data are from specific populations of flies; consequently, our results can only be safely applied to those specific populations. Extrapolating host range results from a single population to all populations of a species is a form of pseudoreplication that can lead to failures in host range predictions (Hopper *et al.* 2005)

Power analyses. We did not use power analyses as discussed by Hoffmeister (2005) in our host range tests. An *a priori* power analysis is useful for predicting the necessary sample size for a test if variability is known (Zilahi-Balogh *et al.* 2005). However, since we rarely knew variability beforehand, we simply continued to increase sample sizes in our tests until standard errors of the means dropped to reasonable levels.

Hoffmeister's (2005) recommendations concerning the use of power analyses to assess the probability of falsely accepting the null hypothesis of "no effect" were not particularly applicable to the kinds of host range tests we did with phorid flies– this was because rates of attraction and parasitism were always very different between target and non-target hosts. Furthermore, if critical aspects of host specificity had been similar enough that they could not be easily resolved statistically, then we would have simply accepted the null hypothesis that no difference existed. We would not have worried whether parasitization rates may have actually been slightly different because they would still have been similar enough to have caused serious concern about the safety of releasing a particular biological control agent in the field.

Hoffmeister's (2005) recommendations concerning power analyses, however, are highly applicable to the assessment of impacts of biological control agents on field populations of target and non-target organisms. In the case of field impacts, it is important to know what power the statistical tests had to resolve differences when no statistical difference was found. This is exactly the problem faced when evaluating the field impacts of *P. tricuspis* on imported fire ants and other ant competitors (Morrison & Porter 2005a). Morrison & Porter (2005a) dealt with the problem by reporting what percent of the mean that two standard errors were. This was done on the assumption that means two standard errors apart would normally be statistically detectable. Power analyses probably provide a more effective way of providing this information.

CONCLUDING REMARKS

The model systems around which many of the general ideas about biological control are framed depart substantially from the phorid–fire ant system in terms both of the enemy and the victim. Conceptually, the decapitating fly – fire ant system resembles host-specific leaf miners and a woody plant host. However, *Pseudacteon* flies are likely to be more host specific than their herbivorous counterparts because the chemical cues they use for host discovery are under selection to be highly distinct among ants for reasons of close physical competition. Ants are mobile, dangerous targets for an attacking fly and the behavior and mechanics of inserting an egg into an armored predaceous host surrounded by aggressive sisters adds additional potential causes for specialized behaviors and morphology in these phorids. Add to these features the likelihood of internal defenses against phorid larvae and it is not surprising that *Pseudacteon* flies exhibit striking host specificity. By contrast the parasitoids of the eggs, larvae and pupae of Lepidoptera, for example, face many fewer challenges that might be solved

by evolving increased specialization. Many practical and theoretical similarities and distinctions of this system and other systems need to be further explored.

CONCLUSIONS

Host range testing is essential because it allows scientists to predict the potential target and non-target impacts of new biological control agents prior to their release in the field. Information about potential impacts, both positive and negative, permits a reasoned decision about whether the likely benefits of releasing a particular agent clearly outweigh the potential problems. The papers in this session and recent books on the subject have set out a number of important procedures and principles that applied to our work with fire ant decapitating flies and to host range testing generally. We would like to emphasize how important it is to do a thorough review of the literature concerning the biology of a prospective agent, the target host, and organisms related to the agent and hosts. We found that biotypes and cryptic species can have different host ranges both as related to target and non-target species; consequently, it is important that biological control practitioners consider this when conducting their tests. We agree that host range tests should be conducted using methods that initially maximize the probability of attack on non-target species. These methods will vary depending on the agent being tested. We attempted to maximize this probability by testing congeners, using small test chambers, using no-choice tests, testing flies of all ages, testing flies in groups, and using both experienced and naïve flies. Good experimental design that uses appropriate controls, randomization, and replication allows valid interpretations to be drawn. Finally, we want to emphasize the need for post-release host range monitoring. Post-release monitoring is important because it verifies the validity of the prerelease testing procedures and provides data that facilitate the release of future biological control agents.

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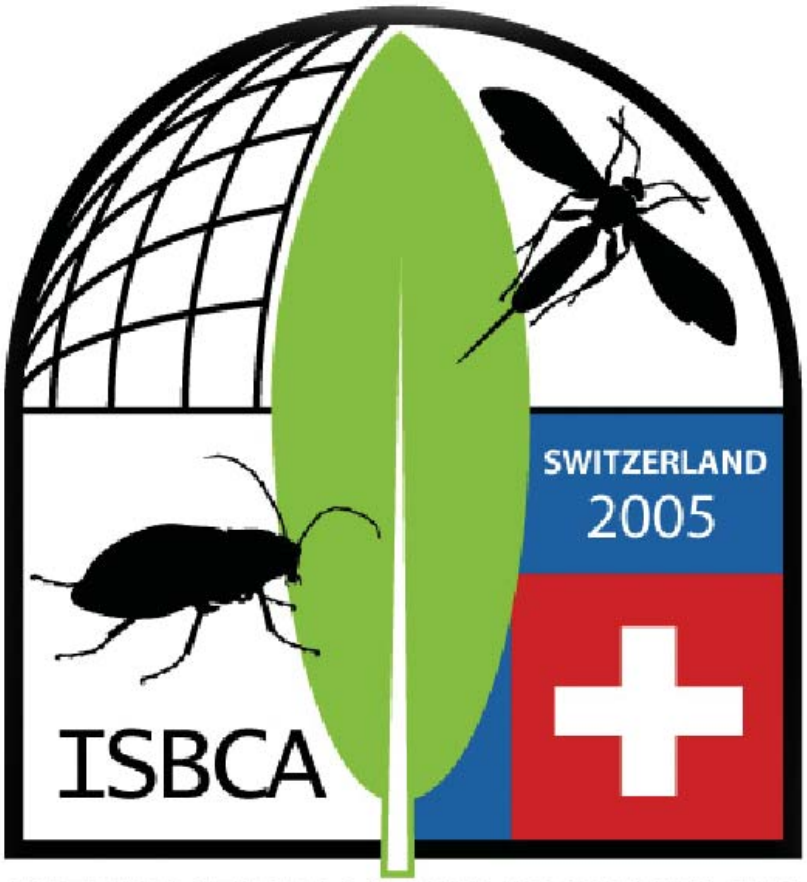

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