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Authors: Vazquez, Ricardo J., and Porter, Sanford D.

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RE-CONFIRMING HOST SPECIFICITY OF THE FIRE ANT DECAPITATING FLY *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE) AFTER FIELD RELEASE IN FLORIDA

RICARDO J. VAZQUEZ[1,2]* AND SANFORD D. PORTER[1]
[1]Center for Medical, Agricultural & Veterinary Entomology, USDA-ARS
P.O. Box 14565, 1600 SW 23rd Dr., Gainesville, FL 32604, USA

[2]University of Florida, Department of Entomology & Nematology, P.O. Box 110620, Gainesville, FL 32611, USA

*Author for correspondence

Critics of biological control programs have argued that there is a lack of post-release monitoring on nontarget effects arising from released exotic insects. Howarth (1991) stated that negative environmental impacts of biological control introductions have not been well documented. Similarly, others have complained that releases of nonindigenous species on target organisms have led to reduction in populations of nontarget species due to inappropriate protocols on host specificity of these nonindigenous species (Barron et al. 2003; Civeyrel & Simberloff 1996; Hopper 2001; Howarth 1991; Secord & Kareiva 1996; Simberloff & Stiling 1996a, b). However, in spite of these criticisms the biocontrol community appears to have a good record of environmental safety (Lindgren 2003; McEvoy et al. 1991). Similarly, Pemberton (2000) analyzed works dealing with 117 natural enemies of 55 weed species and found that only 1 natural enemy completes development in a nontarget plant. A significant problem appears to be that biocontrol practicioners have not always done an adequate job of documenting the post establishment host specificity of organisms that they release.

However, this problem is beginning to be rectified. For example, post-release monitoring has been done for releases of the chrysomelid beetle *Galerucella calmariensis* on purple loosestrife *Lythrum salicaria* (in Michigan: Landis et al. 2003; in Canada: Lindgren 2003; in Oregon: Schooler et al. 2003), the fungal pathogen *Neozygites floridana* on the cassava green mite *Mononychellus tanajoa* in West Africa (Hountondji et al. 2002), the parasitoid wasp *Trichogramma brassicae* on the European corn borer *Ostrinia nubilalis* in Switzerland (Kuske et al. 2003), a South American mirid *Eccritotarsus catarinensis* on the waterhyacinth *Eichhornia crassipes* in South Africa (Coetzee et al. 2003), the rubber vine moth *Euclasta whalleyi* on the rubber vine *Cryptostegia grandiflora* in Australia (Cruttwell McFadyen et al. 2002), the tephritid fly *Acinia picturata* on the exotic weed *Pluchea odorata* in Hawaii (Alyokhin et al. 2001), and the melaleuca weevil *Oxyops vitiosa* on *Melaleuca quinquenervia* in Florida (Paul Pratt, pers. comm.). All of these studies have found minimal or no non-target effects.

The host ranges of phorid decapitating flies in the genus *Pseudacteon* have been studied extensively prior to field releases as self sustaining biocontrol agents of imported fire ants (Folgarait et al. 2002; Gilbert & Morrison 1997; Morrison & Gilbert 1999; Porter 1998, 2000; Porter & Alonso 1999; Porter & Gilbert 2004; Vazquez et al. 2004). *Pseudacteon tricuspis* Borgmeier flies were successfully established on red imported fire ant (*Solenopsis invicta* Buren) populations at eight sites in North Florida (1997-1999: Porter et al. 2004). In the fall of 2003, host specificity of *P. tricuspis* was tested in the field and results demonstrated that these phorid flies had no attraction to non-host organisms including native fire ants (Morrison & Porter 2004). These results are consistent with predictions from quarantine laboratory tests (Gilbert & Morrison 1997; Porter & Alonso 1999) and field tests in South America (Porter 1998) prior to its release in the United States.

A second phorid fly species, *Pseudacteon curvatus* Borgmeier from Formosa, Argentina, was released in Florida to control populations of red imported fire ants (Vazquez et al. 2005). The *P. curvatus* flies were collected attacking *S. invicta* fire ants 35 km NW of Formosa, Argentina by SDP and J. A. Briano (October 2001). *Pseudacteon curvatus* is a small decapitating fly that normally parasitizes small red imported fire ant workers. Quarantine-based host specificity testing predicted that this Formosa biotype was highly host-specific to *S. invicta* and that nontarget effects to the native fire ants, *Solenopsis geminata* (Fab.) and *Solenopsis xyloni* McCook would be minimal to non-existent (Vazquez et al. 2004). *Pseudacteon curvatus* was first successfully released and established in Florida at Whitehurst Farm, 15 mi SW of Gainesville, FL in the spring of 2003 (Vazquez et al. 2005). The objective of this paper is to document the host specificity of established field populations of the Formosa biotype of *P. curvatus*.

Field observations of host specificity were made in October 2003 between 1300 and 1530 EST, when the temperatures were >24°C. We tested the attraction of established *P. curvatus* flies to 15 species of non-*Solenopsis* ants: *Aphaenogaster miamiana* Wheeler (0.8-0.9 mm head width, 0.2 g of workers used), *Aphaenogaster* c.f.

carolinensis Wheeler (0.7 mm, 0.7 g), *Camponotus floridanus* (Buckley) (2.2 mm, 4 g), *Camponotus impressus* (Roger) (0.7-0.8 mm, 0.6 g), *Crematogaster minutissima* Mayr (0.6 mm, 2 g), *Crematogaster pilosa* Emery (0.7-0.9 mm, 2 g), *Cyphomyrmex rimosus* (Spinola) (0.6 mm, 0.2 g), *Dorymyrmex bureni* (Trager) (0.7-0.9 mm, 0.3 g), *Forelius pruinosus* (Roger) (0.5 mm, 0.3 g), *Linepithema humile* Mayr (0.6 mm, 2 g), *Odontomachus brunneus* (Patton) (1.8 mm, 0.4 g), *Pheidole dentata* Mayr (0.6 mm minors, 1.2 mm majors, 0.6 g), *Pogonomyrmex badius* (Latreille) (2.1-2.4 mm, 1.4 g), *Pseudomyrmex pallidus* (F. Smith) (0.6 mm, 0.1 g), *Trachymyrmex septentrionalis* (McCook) (0.8-1.0 mm, 0.2 g), and 6 colonies of *S. invicta* (0.6-1.4 mm, 1.5 g) workers. In the laboratory, *P. curvatus* successfully parasitizes *Solenopsis* ants with head widths of 0.6-1.1 mm (median of 0.74 mm; Morrison et al. 1997 and SDP unpublished data). All ant species used in these tests were collected near Gainesville, Florida (September 2003).

Trays with the 15 non-*Solenopsis* ants were set out first. Trays were 40 × 26 × 8 cm in size, with the inside coated in Fluon (AGC Chemicals Americas Inc., Bayonne, NJ), and contained only one species of ant. We conducted field observations in a 10 × 10 m shady area in one of Whitehurst Farm's well managed pastures (220 ha). The non-*Solenopsis* ants were then removed after 30 min and replaced with the 6 trays of *S. invicta*. At the conclusion of 30 min, the *S. invicta* trays were replaced with the 15 trays of non-*Solenopsis* ants to determine if the flies originally attracted from the *S. invicta* trials would exploit the other genera in the absence of its primary host (no-choice). Established *P. curvatus* flies observed hovering in attack mode over each tray were collected at 5 min intervals for 30 min. All flies were aspirated with an Allen-type double chamber aspirator and retained in vials until the conclusion of each 30 min trial when they were identified to species using a hand lens. Aspiration of flies normally does not change attack behavior once flies are released (Morrison et al. 1997). Therefore, the released flies readily resumed attacking red imported fire ants. Collection and identification for presence of *P. curvatus* flies was necessary since *P. tricuspis* flies were present at the study site from a release in Gainesville, Florida, in the summer and fall of 1997 (Porter et al. 2004). Flies captured during observations were then released prior to setting up additional trays. These methods were replicated on two consecutive days.

Further tests of *P. curvatus* host specificity were conducted with five trays of *S. invicta* and five trays of the native fire ant, *S. geminata*. Each tray contained 2 g of workers and 2 g of brood. As described above, the five trays of *S. geminata* were set out first for 30 min. *Solenopsis geminata* trays were then removed and replaced with the *S. invicta* trays and these trays were observed for 30 min. At the conclusion of 30 min, the five trays of *S. invicta* were replaced again with the five trays of *S. geminata* for an additional 30 min. Attacking flies were collected at 5 min intervals as described above. These methods were replicated on two days (five days apart) at the same site mentioned above.

The *P. curvatus* flies were not attracted to any of the 15 non-*Solenopsis* genera during the sequential series trials over the two days (Table 1). However, the flies were readily attracted to *S. invicta* (99 on day 1 and 38 on day 2, Table 1). As is normal, these flies hovered above their host, oriented themselves to workers, and readily struck the thorax of workers in an attempt to oviposit in the ants. When the six *S. invicta* trays were removed and replaced again with the 15 trays of non-*Solenopsis* ants, *P. curvatus* flies were not observed hovering over any of the non-*Solenopsis* trays. *Pseudacteon curvatus* flies were present at all six *S. invicta* trays during the trials.

In the *S. invicta* versus *S. geminata* trials, *P. curvatus* flies were not observed hovering or attacking over *S. geminata* during the first day and only 2-4 flies were observed hovering on the second day (Table 1). Flies collected above the native fire ants generally hovered briefly without attacking. Only one fly attempted to oviposit, but it flew away immediately after without returning. In quarantine tests, this biotype would occasionally attack *S. geminata* workers but attacks were never successful (Vazquez et al. 2004). *Pseudacteon curvatus* flies were present at all five *S. invicta* trays during the first day and present at four of five trays on the second day. *Pseudacteon curvatus* flies were present at none of the five *S. geminata* trays during the first day and at 1 of 5 and 3 of 5 trays on the second day (Table 1).

Field-established *P. curvatus* individuals were attracted to *S. invicta* over *S. geminata* by a ratio of about 30 to 1 (119 to 4 total flies, Table 1). These results were better than results predicted from quarantine tests where *P. curvatus* hovered over *S. invicta* versus *S. geminata* at a ratio of 1.3 to 1 in no-choice tests (Vazquez et al. 2004). Perhaps this difference was because *P. curvatus* flies in the laboratory tests were confined in small test containers leading to higher rates of hovering. Furthermore, attacks on *S. geminata* were very rare to non-existant in the field confirming laboratory choice tests where attack rates were 16 times higher for females hovering over *S. invicta* than for flies hovering over *S. geminata* (7.02 ± 1.41 (mean ± SE) versus 0.44 ± 0.28 attacks/min, respectively; Vazquez et al. 2004). We demonstrated in quarantine tests (no-choice and choice) that the Formosa biotype of *P. curvatus* does not complete development in *S. geminata* (Vazquez et al. 2004).

Post-release populations of *P. curvatus* were not attracted to any of the 15 non-host ant genera. In host-specificity tests with a biotype from Las Flores, Argentina, *P. curvatus* hovered over most

TABLE 1. NUMBER OF *PSEUDACTEON CURVATUS* FLIES COLLECTED HOVERING IN ATTACK MODE OVER NON-HOST ANT SPECIES, NATIVE FIRE ANTS (*SOLENOPSIS GEMINATA*), AND RED IMPORTED FIRE ANTS (*SOLENOPSIS INVICTA*) DURING SEQUENTIAL SERIES OF FIELD TRIALS (SEE METHODS).

Ant species	Flies collected				Trays attacked
	0-10 min	11-20 min	21-30 min	Total	
S. invicta vs 15 non-host genera (day 1)					
All 15 genera	0	0	0	0	0/15
S. invicta	14	56	29	99	6/6
All 15 genera	0	0	0	0	0/15
S. invicta vs 15 non-host genera (day 2)					
All 15 genera	0	0	0	0	0/15
S. invicta	7	14	17	38	6/6
All 15 genera	0	0	0	0	0/15
S. invicta vs *S. geminata* (day 1)					
S. geminata	0	0	0	0	0/5
S. invicta	28	20	18	66	5/5
S. geminata	0	0	0	0	0/5
S. invicta vs *S. geminata* (day 2)					
S. geminata[a]	0	1	1	2	1/5
S. invicta	14	16	23	53	4/5
S. geminata[b]	1	3	0	4	3/5

[a]No oviposition attempts were observed.
[b]Only one oviposition attempt was observed.

of 19 non-host genera in quarantine conditions (Porter 2000); however, they generally hovered without attacking and no parasitism occurred in any of the 19 non-host genera (Porter 2000). Results from this study demonstrate that host specificity of *P. curvatus* is restricted to *S. invicta* and poses no realistic threat to the congener *S. geminata* or ants in other genera.

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SUMMARY

Post-release monitoring confirms that the Formosa biotype of *P. curvatus* is not attracted to non-*Solenopsis* ants. Flies were attracted to the native fire ant, *S. geminata*, at very low rates (<5% of that with *S. invicta*) but virtually no oviposition attempts were observed. Overall results were consistent with laboratory predictions except attraction rates to nontarget fire ants in the field were much lower than in small laboratory test chambers.

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