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DISTRIBUTION OF *PSEUDACTEON CURVATUS* AND *PSEUDACTEON TRICUSPIS* (DIPTERA: PHORIDAE) IN ARKANSAS

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ABSTRACT

From 1995 to 2009, four *Pseudacteon* species were released in the U.S. with 3 species, *P. curvatus*, *P. tricuspis*, and *P. obtusus* released in Arkansas. To determine *Pseudacteon* establishment and expansion, sticky traps were used to monitor phorid fly species at and near 10 release sites, in counties bordering neighboring states, and along regional transects. *Pseudacteon* flies were captured in 16 Arkansas counties: Ashley, Chicot, Clark, Desha, Drew, Hempstead, Howard, Little River, Montgomery, Nevada, Perry, Phillips, Pike, Polk, Sevier, and Union. *Pseudacteon curvatus* was found in areas far from release sites, suggesting dispersal from neighboring states. The range of *P. tricuspis* evidently also expanded from its initial release sites in southern Arkansas.

Key Words: biological control, phorid fly, *Solenopsis invicta*, parasitoid

RESUMEN

De 1995 hasta el 2009, cuatro especies de *Pseudacteon* fueron liberadas en los Estados Unidos con 3 de ellas, *P. curvatus*, *P. tricuspis* y *P. obtusus*, liberadas en el estado de Arkansas. Para determinar el establecimiento y expansión de *Pseudacteon*, se usaron trampas pegajosas para monitorear las especies de fòridos en y alrededor de los 10 sitios donde fueron liberadas, en condados fronterizos con los estados vecinos, y a lo largo de las lineas regionales. Las moscas *Pseudacteon* fueron capturadas en los siguientes 16 condados de Arkansas: Ashley, Chicot, Clark, Desha, Drew, Hempstead, Howard, Little River, Montgomery, Nevada, Perry, Phillips, Pike, Polk, Sevier y Union. *Pseudacteon curvatus* fue encontrada en áreas lejos de los sitios donde fue liberada, que indica que se disperso de los estados vecinos. Evidentemente, el rango de *P. tricuspis* tambien se expandió de los sitios donde fue liberada inicialmente en el sur de Arkansas.

Pseudacteon phorid flies parasitize and kill their fire ant host (*Solenopsis invicta* Buren, *S. richteri* Forel and their hybrid). Due to their high host specificity (Folgarait et al. 2002; Gilbert & Morrison 1997; Morrison & Gilbert 1999; Porter 1998a, 2000; Porter & Alonso 1999; Porter & Gilbert 2004; Vazquez et al. 2004), several *Pseudacteon* spp. have been introduced from South America as classical biological control agents against imported fire ants. While internal development of the fly larvae eventually decapitates and kills individual ants (Porter et al. 1995a; Consoli et al. 2001), perhaps the most important effect is the disruption of the foraging behavior of the ants (Feener & Brown 1992; Orr et al. 1995; Porter et al. 1995b; Mehdiabadi & Gilbert 2002), which leads to a decrease in food uptake and a decline in colony health (Folgarait & Gilbert 1999). Four *Pseudacteon* species were released in the U.S. from 1995 to 2009: *P. curvatus* Borgmeier, *P. litoralis* Borgmeier, *P. obtusus* Borgmeier, and *P. tricuspis* Borgmeier. Each species fills a different niche, in terms of diurnal activity (Pesquero et al. 1996), seasonal occurrence (Fowler et al. 1995; Folgarait et al. 2003) and preferred size of host (Campiolo et al. 1994); all complementing traits for control of *S. invicta* and *S. richteri* (Morrison

et al. 1997; Porter 2000; Folgarait et al. 2002, 2005).

Pseudacteon flies use semiochemicals to locate their ant hosts (Orr et al. 1997; Vander Meer & Porter 2002; Morrison & King 2004; Chen & Fadamiro 2007) and then hover over the ants before a rapid aerial attack (Morrison & Porter 2005). Within a period of an hour a female fly can make up to 120 oviposition attempts (Morrison et al. 1997), before either tiring or being captured and killed by the ants (Porter 1998b). A single egg laid in the thorax develops through 3 instars before decapitating the ant's head, which is then used as a pupal case (Pesquero et al. 1995; Porter et al. 1995a).

The first release of *P. tricuspis* occurred in Texas in 1995 (Gilbert 1996), and was unsuccessful due to unfavorable conditions (Vazquez et al. 2006). The first successful release of *P. tricuspis* in northern Florida was in 1997 (Porter et al. 1999). *Pseudacteon* spp. have been released in 11 southern states: Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, and Texas (Porter et al. 1999; Graham et al. 2003; Williams & deShazo 2004; Parkman et al. 2005; Thead et al. 2005; Henne et al. 2007; Weeks & Callcott

2008). *Pseudacteon curvatus*, *P. obtusus*, and *P. tricuspis* have been released in Arkansas and in adjacent states except Missouri (Clemons et al. 2003; Weeks & Callcott 2008).

Dispersal rates for *Pseudacteon* spp. are variable and the majority of flies disperse only a few hundred meters (Morrison et al. 1999). However, some flies in each generation are known to travel 2 to 4 km or more (Porter et al. 2004) and populations of flies expanding on average 74 km over a period of 3.5 years (Porter 2010).

Monitoring of *Pseudacteon* spp. is achieved with a variety of methods: actively through direct collections of flies at disturbed ant colonies with either manual or electrical stimulation (Barr & Calixto 2005; Morrison & Porter 2005), or passively by trapping with a sticky trap (Puckett et al. 2007). Until the current study, monitoring of these species in Arkansas had been concentrated at and near release sites to determine *Pseudacteon* establishment. The objective of this study was to determine the distribution of *Pseudacteon* spp. in Arkansas through wider-scale monitoring.

MATERIALS AND METHODS

Trap Design

To determine presence or absence, passive trapping was used based on a modified version of a PTS (pizza tri-stand) sticky trap (Puckett et al. 2007). The modified Puckett trap (Fig. 1) consisted of a pizza tri-stand (Polyking® No. 20431) covered in Tanglefoot®, glued to the flat side of an inverted portion cup (Dart® No. 100PC), which was hot glued to the underside of a plastic cup lid (Dart® No. 8JL). This device was placed in the center of the bottom half of a plastic Petri dish (150 by 15 mm). The surfaces of the portion cup and the inner lip of the Petri dish were coated

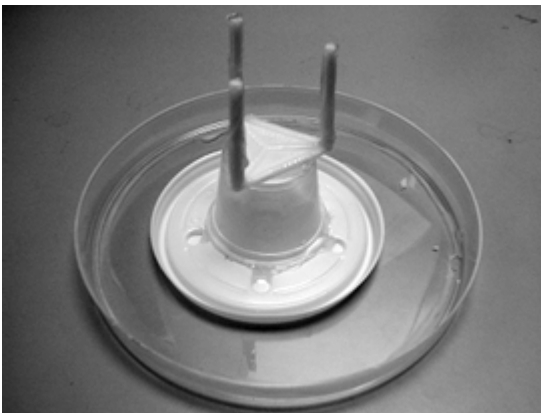


Fig. 1. A modified Puckett trap.

with Fluon®, to prevent ants from climbing up the trap or out of the Petri dish.

One trap was placed per location by first locating a mound of substantial size and activity. The mound was then disturbed, by kicking it over creating a flat surface on which the Petri dish was placed. As ants climbed into the Petri dish, a few ants were crushed by hand to induce alarm pheromone release and the trap was placed in the center of the Petri dish. A brightly colored pin flag (91 cm long) was positioned alongside the trap. Global positioning system (GPS) coordinates were recorded for each location, and a corresponding number written on the lid of the trap.

Traps were retrieved 20 to 24 h after placement. At time of retrieval, an 8 oz expanded polystyrene foam cup (Dart® No. 8J8) was placed over the trap, and the lid was snapped in place. The cup prevented damage and contamination to the sticky portion of the trap.

Sampling at Release Locations

Fourteen releases of *Pseudacteon* spp. were made in Arkansas from 1998 to 2009 (Table 1). Sampling along transects in the cardinal directions from the release sites began in 2002 (Pike Co.) and 2004 (Miller Co.). In 2009, the Miller, Perry, Pike, and Sevier County release sites were reevaluated for this study to confirm establishment of *Pseudacteon* spp. A 1.6-km interval was used between traps along each transect placed in Pike, Miller, and Sevier Counties, and at 0.8-km intervals in Perry County. Transects were determined by locating roads and highways on aerial maps that radiated out from the release site in north, south, east, and west directions. One modified Puckett trap was placed at each sampling location.

Sampling Bordering Counties

Sampling was intended to monitor spread of established populations of *Pseudacteon* spp. in bordering counties/parishes of neighboring states of Louisiana (Henne et al. 2007), Mississippi (Thead et al. 2005), and Tennessee (Graham et al. 2003; Parkman et al. 2005; Weeks & Callcott 2008), and sampling packages were sent to University of Arkansas Cooperative Extension Service County Agents in imported fire ant infested counties in eastern and southern Arkansas in the early summer of 2009. Each package contained four modified Puckett traps, latex gloves, and an information sheet. Instructions were to place the traps during the summer months, as previously described, at 4 locations within the county. Deployed traps were returned from 4 bordering counties: Columbia, Lafayette, Phillips, and St. Francis. Geographic coordinates were recorded on the instruction sheet with the number of the corresponding trap.

TABLE 1. HISTORY OF *PSEUDACTEON* SPP. RELEASES IN ARKANSAS, 1998-2009.

| Date | County | Species | Number Released |
|----------------------------|-----------|---------------------|---------------------|
| 1998, Jul-Aug ¹ | Drew | <i>P. tricuspis</i> | 1,350 |
| 2002, May ² | Pike | <i>P. tricuspis</i> | 3,000 |
| 2002, Oct ² | Bradley | <i>P. tricuspis</i> | 1,200 |
| 2003, Sep ² | Bradley | <i>P. tricuspis</i> | 1,500 |
| 2004, May ² | Miller | <i>P. tricuspis</i> | 2,580 |
| 2005, May ² | Sevier | <i>P. tricuspis</i> | 4,300 |
| 2005, Oct ² | Clark | <i>P. curvatus</i> | 8,500 ³ |
| 2006, Sep ² | Perry | <i>P. curvatus</i> | 15,816 ⁴ |
| 2007, Sep ² | Perry | <i>P. tricuspis</i> | 1,900 |
| 2008, Jun ² | Jefferson | <i>P. tricuspis</i> | 120 |
| 2008, Oct ² | Polk | <i>P. obtusus</i> | 3,200 ⁵ |
| 2009, May ² | Grant | <i>P. curvatus</i> | 25,296 ⁶ |
| 2009, Jun ² | Jefferson | <i>P. tricuspis</i> | 120 |
| 2009, Sep ² | Garland | <i>P. tricuspis</i> | 360 |

¹Release made by Lynne Thompson, University of Arkansas Monticello.

²Release made by Kelly Loftin, University of Arkansas Cooperative Extension Service.

³Number released based on 35.3 g *S. invicta* workers, 800/gram and 30% parasitism.

⁴Number released based on 65.9 g *S. invicta* workers, 800/gram and 30% parasitism.

⁵Number released based on 23.7 g *S. invicta* workers, 450/gram and 30% parasitism.

⁶Number released based on 105.4 g *S. invicta* workers, 800/gram and 30% parasitism.

Sampling Along Regional Transects

Due to possible expansion of *Pseudacteon* spp. from neighboring states, sampling transects were identified and mapped in 3 regions: western, southeastern, and southwestern Arkansas (Fig. 2). Transects began at the state line of the adjacent state and traveled inward, with modified Puckett traps placed at 3-mile intervals and GPS coordinates recorded for each trap. Sampling was conducted for 36 h along transects from each region between 19 Sep and 3 Oct 2009.

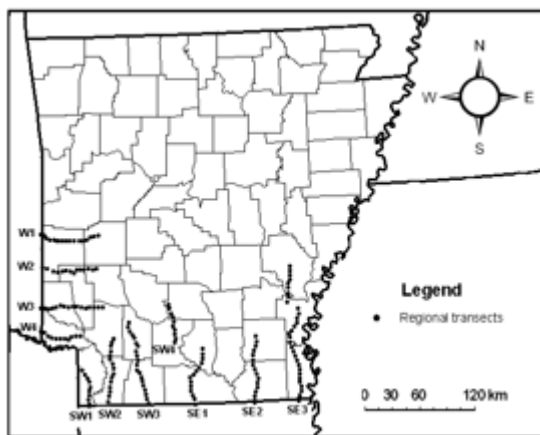


Fig. 2. Regional transects in Arkansas (SE1, SE2, SE3, SW1, SW2, SW3, SW4, W1, W2, W3, W4) (ESRI Inc. 2009).

Phorid Fly Identifications

Upon retrieval, modified Puckett traps were taken to the University of Arkansas laboratory (Fayetteville, AR) and GPS coordinates logged into Google Earth™. Traps were examined under a dissecting microscope for presence of *Pseudacteon* spp. The sticky portion of the trap was sprayed with liquid degreaser (Goo Gone™) if fly removal was necessary. Species were identified based on the morphology of the female ovipositor (Porter & Pesquero 2001). Voucher specimens of *P. curvatus*, *P. tricuspis*, and *P. obtusus* were obtained from laboratory specimens and the phorid fly rearing facility in Gainesville, FL for identification of male and female flies. Male *Pseudacteon* spp. are currently difficult to identify through use of keys (Morrison et al. 1997; Porter & Pesquero 2001).

RESULTS AND DISCUSSION

Release Locations

Traps placed along transects radiating from the phorid fly release sites yielded surprising results. Of the 20 traps retrieved from Sevier County, no *Pseudacteon* spp. were caught. Traps from Miller, Perry, and Pike Counties captured *Pseudacteon* spp. Along the Miller County release site transects, *P. curvatus* was captured at 3 locations north of Texarkana, in the southeastern part of Little River County (Fig. 3). However, this was unexpected because the phorid fly species released in Miller County in 2004 was *P. tricuspis*

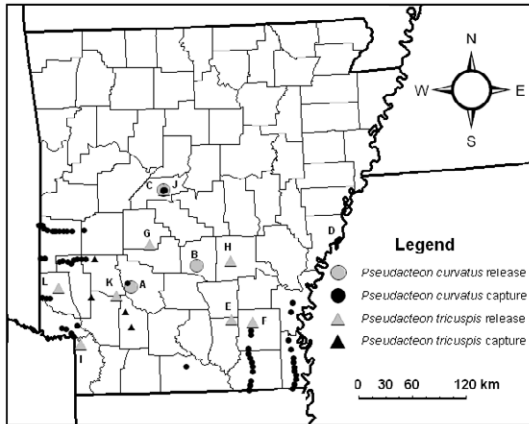


Fig. 3. *Pseudacteon curvatus* release sites in (A) Clark Co., (B) Grant Co., (C) Perry Co., and 2009 capture locations including (D) Phillips Co., Arkansas. *Pseudacteon tricuspis* release sites in (E) Bradley Co., (F) Drew Co., (G) Garland Co., (H) Jefferson Co., (I) Miller Co., (J) Perry Co., (K) Pike Co., (L) Sevier Co., Arkansas and 2009 capture locations (ESRI Inc. 2009).

(Fig. 3, I on map). While possible, it is unlikely that these captures resulted from cross contamination of the 2 species at the rearing facility. Several factors are in place to prevent cross contamination including parasitization of the ants in separate rooms and lack of available ants of preferred host size. Furthermore, voucher sampling is conducted to insure parasitization by the correct species (Amy Croft, Florida Department of Agriculture and Consumer Services, personal communication). A plausible explanation could be the expansion of *P. curvatus* from released and established populations in the bordering states of Louisiana, Oklahoma, and Texas (Weeks & Callcott 2008; Anne-Marie Callcott, USDA-APHIS, personal communication). Similar results were observed along the northeast transect of the Pike County release site; capture of a *Pseudacteon* fly (*P. curvatus*) different from the species originally released (*P. tricuspis*). Due to the proximity (5 km) with the Clark County release site (Fig. 3A), it is possible that this capture was from movement of *P. curvatus* from Clark County, although revaluation of Clark County was not conducted in 2009. In Perry County, capture of *P. curvatus* was recovered at the initial release site and at locations to the south (0.5 km) and east (2.2 km).

Bordering Counties

Puckett traps from Columbia, Lafayette, and St. Francis Counties were devoid of *Pseudacteon* flies, although all traps returned from Phillips County on the Mississippi border captured *P. cur-*

vatus. Phillips County is the one of 2 counties in Arkansas (Crittenden Co. the other) currently known to have only *S. richteri* and no record of *S. invicta* (Robert Vander Meer, USDA-ARS, personal communication). Sampling locations in Phillips Co. were located on the western levee of the Mississippi River, northwest of Friars Point, MS (Fig. 3D), adjacent to counties in Mississippi and Tennessee with known *S. richteri* populations (Streett et al. 2006; Oliver et al. 2009). The high number of *P. curvatus* found on 1 trap (~172) implies sufficient colonies of *S. richteri* to support *P. curvatus* populations.

Regional Transects

A total of 176 modified Puckett traps were placed along transects in western, southeastern, and southwestern Arkansas (Fig. 2). In the western region of transects, 28 traps contained *P. curvatus*, and 2 contained *P. tricuspis* (Fig. 3). Of the traps that captured *P. tricuspis*, one was located 29.5 km west of the *P. tricuspis* release in Pike County, and the other was 46 km northwest of the release site. All 4 transects in this region included captures of *P. curvatus*. On the 2 northerly routes (W1 and W2), traps with *P. curvatus* were found at regular intervals with the most easterly capture 54 km from the Arkansas/Oklahoma state line. The remaining 2 transects (W3 and W4) also had captures. Transect (W3) picked up *P. curvatus* 13 km from the Arkansas/Oklahoma border. The 3 traps from the most southerly transect (W4) that contained *P. curvatus* were located near the northern section of the Miller County release site transect. This directional pattern of recoveries supports the hypothesis of immigration from confirmed *P. curvatus* populations in Le Flore County, Oklahoma (Weeks & Callcott 2008) approximately 24 km northwest of Mena, Arkansas. Prevailing winds generally do not correlate with dispersion patterns (Morrison et al. 2000), as flies move close to the ground where wind is reduced.

Pseudacteon curvatus but not *P. tricuspis* was trapped along the southeastern region transects. *P. curvatus* was found at 1 location in Union County, north of El Dorado, and on 12 traps along each of the other transects (SE2 and SE3) to the east (Fig. 3). From the 2 easterly transects (SE2, SE3), traps with *P. curvatus* were found 91 km north of the Arkansas/Louisiana border and 65 km west of the Arkansas/Mississippi border. This distribution in Arkansas is expected based on collections of *P. curvatus* in bordering counties along the western side of Mississippi (Adams, Bolivar, Claiborne, Coahoma, Desoto, Jefferson, Tunica, Warren, Washington, Wilkinson), and along Louisiana's northern side (Claiborne, East Carroll, Morehouse, Union, West Carroll) (Anne-Marie Callcott, USDA-APHIS, personal communication).

Pseudacteon tricuspsis was captured at 2 locations along a transect (SW3) of the southwestern region of Arkansas (Fig. 3). One of the traps was located in the northern part of Nevada County, in the city of Prescott, and the other was 18 km to the southeast. Their proximity to the Pike County release site (Fig. 3K), 20 km northwest, may suggest their origin, although *P. curvatus* appeared in no samples taken from the Pike County release site transect. Weather conditions may have played a role in the lack of *Pseudacteon* spp. present on the remaining 55 traps in this region. Temperatures for the region on 3 Oct 2009 ranged from a low of 8°C to a high of 26°C. Temperatures below 20°C inhibit activity of *Pseudacteon* flies (Morrison et al. 1999; Wuellner et al. 2002). On 3 Oct 2009 temperatures were above 20°C for a 7-h period midday (11:00 AM to 6:00 PM CST) and below 20°C for the entire day of 4 Oct 2009, when the traps were retrieved. However, 2 traps located on the transect southeast of Prescott, AR collected *P. tricuspsis*. Weather data suggests similar conditions for the Prescott, AR area, although slightly warmer temperatures (17°C) were recorded for an additional 3.5 h on the morning of 4 Oct 2009.

Of the traps that collected *Pseudacteon* spp., no traps contained both species. Perhaps because *P. tricuspsis* is reliant on larger ants, *P. curvatus* is able to establish more readily where *P. curvatus* and *P. tricuspsis* overlap, and thus were not detected if present in low densities (Gilbert et al. 2008). Another factor for the lack of both species in the trap may be due to the modification of the Puckett trap. The 2 differences in the traps design were the attractant used and the placement of the trap. Traps were placed on a disturbed mound with live ants whereas Puckett traps were placed in an open area with midden (Puckett et al. 2007). The Puckett trap as originally designed captured more *P. tricuspsis* than *P. curvatus*, although seasonal fluctuations could be a variable (Puckett et al. 2007).

CONCLUSION

Passive *Pseudacteon* trapping with the modified Puckett trap provided advantages over direct collection from disturbed mounds. Because it is deployed quickly, multiple traps can be placed over a large area which allows continuous and simultaneous sampling (Puckett et al. 2007). The manpower needed to achieve similar coverage using observational sampling is cost prohibitive. The addition of the protective cup to the original design allowed longer storage time between collection and examination of the trap, protection of the sticky portion, and reduced contamination. The modification based on disturbed mounds and flouon-coated petri dishes rather than fire ant midden was advantageous in that maintenance of a fire ant colony for collection of midden is no longer

necessary. With this modification, trapped fire ants rather than fire ant midden serve as the *Pseudacteon* fly attractant.

The results suggest establishment and expansion of *P. curvatus* from the release site in Perry County, and *P. tricuspsis* from the release site in Pike County. While limited, the current range of *P. tricuspsis* in Arkansas appeared to be along a narrow 86-km band stretching from northwest Pike County to south central Nevada County, and 25 km west of the release site. The current distribution of *P. curvatus* in Arkansas suggested natural movement from surrounding states. Despite extensive sampling across southern Arkansas, many areas remained unsampled. Additional trapping would provide a better understanding of the distribution of *Pseudacteon* spp. in Arkansas.

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