

**Release and establishment of a fire ant parasitic fly, *Pseudacteon curvatus* and *Pseudacteon tricuspis* (Diptera: Phoridae) in Denton County and the release of *Pseudacteon curvatus* in Red River County in 2007**

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Biological control, combined with conventional chemical methods, are now being used to manage the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in the United States. Classical biological control has the potential to effectively control pest populations and in some cases it is more effective than conventional chemical methods. There are many natural enemies of the red imported fire ant that have been identified in South America (Knutson and Drees 1998). These natural enemies have the ability to reduce the number of fire ant colonies in an area, sustain themselves and spread over time. A parasitic fly in the genus *Pseudacteon* (Diptera: Phoridae) is one natural enemy from South America that attacks fire ants in the genus *Solenopsis*. All of these fly species are host-specific and do not affect any other ants or animals (Porter and Gilbert 2004). This is a logical approach to controlling fire ants, since this fly will provide a sustained pressure that is specific to red imported fire ants.

*Pseudacteon* sp. is a parasitic fly whose larvae develop inside the head of a fire ant worker. The mated female fly lives for several days, so she can seek foraging worker ants in the daytime. Upon finding a foraging worker fire ant, she lays her egg inside the thoracic region of the fire ant worker. After the egg hatches, the larva digests the tissues within the thoracic region of the fire ant worker and then the fly larva moves into the head. The fire ant worker's head falls off and the larva continues to feed on tissues within the head and then pupates inside the head. The other fire ants within the colony will remove the parasitized ant from the colony, so the adult fly will be able to emerge from the head capsule outside the colony and begin seeking new fire ant hosts nearby. The male and female flies will mate after their emergence and then the females will hover over the fire ant workers until her eggs are deposited (Knutson and Drees 1998).

Phorid flies impact fire ant colonies in two ways. First, these flies selectively detect and kill fire ant workers through parasitism. This causes around 2% of the worker ants in the colony to become parasitized. Secondly, host-seeking female phorid flies causes shifting in fire ant foraging behavior. After a fly detects a fire ant worker, the fly hovers over the worker making the worker hide to avoid attack, thereby disrupting day-time surface activities of the worker ants. This causes the ants to spend more time avoiding the flies and less time foraging for food (Porter 1998).

Currently there are five candidate species within the genus *Pseudacteon* that attack fire ants. However, only two species have been released in Texas, *Pseudacteon tricuspis* and *Pseudacteon curvatus*. With *P. tricuspis*, which has been released and established in a

number of release sites in Texas, the sex of the fly is determined by the worker ant head capsule size. Females are only produced in large major worker ants, and fly larvae developing in smaller minor workers became males. However in North Texas, the multiple queen colonies are more prominent, so the average fire ant worker size is small. Therefore, many males and only a few females are produced in polygyne fire ant colonies and often fail to sustain a phorid fly population. Conversely, *P. curvatus* sex ration is not determined by worker ant head capsule size (Morrison and King, 2004). Both *P. tricuspus* and *P. curvatus* have also been released in other southern states such as Florida, South Carolina, Louisiana, Georgia, and Alabama.

This trial was a collaborative effort between Texas Cooperative Extension (TCE) and the United States Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) that supplied the phorid flies for both releases.

## Materials and Methods

The first release site of *P. curvatus* in September 2004 was located on the M.T. Cole Ranch, one mile north of US 380 on the Denton-Wise county line. The release site consisted of a 2 acre plot, in the middle of a 40 acre ranch. The 2 acre release site was subdivided into 8 quarter acre plots. Sixty-three active fire ant mounds were located within the 8 quarter acre plots at GPS coordinates of 33° 16' 08.95500" N and 97 ° 23' 39.82974" W. On April 18 and November 1, 2007, we monitored the spread of the flies using passive “pizza” traps placed approximately every two miles for a total of 20 miles in the north, south, east and west directions from the release site (Puckett, *In press*) (Table 1). The passive traps consisted of a large petri dish containing a smaller petri dish filled with ¼ cup of midden (decomposing fire ants) and an upside down pizza tri-stand covered with Tanglefoot® (Figure 1). The phorid flies are attracted to the midden and perch onto one of the prongs of the pizza stand. The flies become stuck to the trap, which allows the traps to be placed into certain locations for up to 48 hours. We collected the traps after 24 hours and then placed each trap under a microscope so the phorid flies could be properly identified. With the help of volunteers, the traps were placed out in each direction from 11am to 1:00pm with temperatures at 72°F. The traps remained in there respective locations overnight and each one was collected on April 19<sup>th</sup> and November 2<sup>nd</sup> from 12pm-2pm with temperatures at 76°F and 72°F, respectively. These were the final two observations for this location.

Another release of *P. curvatus* was conducted in Red River County (Figure 3). Two different collections and releases were made on a total of 24 mounds. The number of live ants collected and the location of each mound can be found in Table 2. Approximately four grams of fire ants from each mound were collected in separate containers, containing a moistened paper towel at the bottom of the container to prevent dehydration. These fire ants were noticeably more aggressive than those previously collected in the Denton area, so the total collection time was only two hours each week. After the fire ants were collected into the sealed container, a rubber band was placed over the container and all the containers were placed into two pillow cases. The pillow cases were placed into a Styrofoam cooler with a frozen, blue ice block at the bottom. The cooler was shipped overnight to the USDA-APHIS lab in Gainesville, FL. The fire ants were kept in chambers with the flies for 6 days, so the flies would have enough time to parasitize the ants. The parasitized ants were then shipped back to the Red River County Extension Office in Clarksville, TX to be released within the

same afternoon. The first shipment to Gainesville, FL was sent on October 11<sup>th</sup> and the remaining shipment was sent on October 17<sup>th</sup>. Temperatures during the release periods were in the upper 70s, with cloudy skies. Releases were conducted from 3:00-4:00pm on October 19<sup>th</sup> and 26<sup>th</sup>. The releases were made by slightly disturbing the mound using a stick, and then placing the individual container near the original colony. The containers were open and the paper towel was unfolded and placed on top of the colony. No aggressions were observed between ants coming from the colony towards the parasitized ants. The paper towel and containers were removed from the colony when no more ants were observed outside the colony. Since we were able to collect so many fire ants, the USDA-APHIS laboratory saved mounds 14, 15 and 16 for parasitization by *P. tricuspis*. These worker ants were returned on October 25<sup>th</sup> for release onto their original mounds.

The release site for *P. tricuspis* was below the dam of Lake Ray Roberts in Pilot Point, TX (Figure 2). Active mounds were located along the sidewalk and marked with flags. A total of 2,906 flies were released onto 53 mounds. The GPS coordinates were taken for each active mound and releases were conducted daily from 12:00pm-2:00pm from October 16<sup>th</sup> through October 31, 2006. On April 18 and November 1, 2007, “pizza traps” were placed into the release area to determine the establishment of a population. Ten traps were placed in the release area on April 18<sup>th</sup> and 15 traps were placed in the release area on November 1<sup>st</sup> (Table 3). The traps remained in their respective locations overnight and each one was collected on April 19<sup>th</sup> and November 2<sup>nd</sup> from 10:30-11:30 with temperatures at 72°F and 71°F, respectively.

## Results

On October 14, 2005, *P. curvatus* were observed at the M.T. Cole Ranch at the one year observation. In October 2006, the population expanded and we were able to recover flies on the trap 4 miles north and on the trap 4 miles south of the original release site. On April 19, 2007 (Table 4), flies were recovered on the traps 2 and 4 miles east, 2 and 6 miles north and 4 miles west. There were no flies recovered on the south traps. On November 2, 2007 (Table 5), flies were recovered on traps 6 miles north and 6 miles south. There were no flies recovered on the east and west traps.

At this time, we have not monitored the *P. curvatus* release site in Red River County, but plan to observe the site for activity in April and October 2008.

For the 2006 release of *P. tricuspis*, we observed the release site for flies on April 18 and November 1, 2007. On April 19, 2007 (Table 6), two flies were recovered on trap 5 and one fly was recovered on trap 10. On November 2, 2007 (Table 7), one fly was recovered on trap 8 and one fly was recovered on trap 14. We plan to continue monitoring this site for the next two years.

## Discussion

Little is known about the favorable environmental conditions *P. curvatus* and *P. tricuspis* must have in order to establish a population. It is believed that *P. curvatus* is better adapted to survive in polygyne red imported fire ant populations found in North Texas and perhaps even farther north in Red River County. However, we were able to successfully release *P. tricuspis* at Ray Roberts Park. This is the second release of this fly species but first

successful establishment of the fly in North Texas. We plan to monitor the expansion of the phorid population at Ray Roberts Park in April and October 2008 using the passive “pizza” trap for 20 miles in each of the four directions. Also “pizza” traps will be placed in the release area in Red River County in April and October 2008. The continued monitoring of both release sites will help in determining establishment and spread of the flies into new areas.

By introducing and establishing these two parasitic phorid fly, we are providing a natural biological stress on fire ant colonies. Hopefully, this will allow native ants to better compete with this introduced species and regain their territories. As the native ants reclaim their territories, it is hoped that the number of red imported fire ant colonies will decrease. The potential of sustainable fire ant population suppression will thereby reduce the \$1.2 billion annual impact of *S. invicta* in Texas and the need for insecticides for their control will be reduced.

**Figure 1.** Passive “pizza” trap used to monitor the spread of *P. curvatus* from the original release site at the M.T. Cole Ranch on the Denton/Wise county border.



**Figure 2.** Release site of *P. tricuspis* along the sidewalk below the dam of Lake Ray Roberts, Pilot Point, TX, with flags marking active fire ant mounds.



**Figure 3.** Release site of *P. curvatus* and *P. tricuspis* on a 50 acre site in Clarksville, Red River County, TX.



**Table 1.** Coordinates of each passive “pizza” trap placed 20 miles north, south, east and west of the original release site at the M.T. Cole Ranch, in order to detect the spread of *P. curvatus* used on April 18 and November 1, 2007.

<b>Trap</b>	<b>North</b>	<b>South</b>	<b>East</b>	<b>West</b>
<b>1</b>	33 18 32.2 N 97 23 27.1 W	33 16 12.4 N 97 23 66.5 W	33 16 37.24 N 97 20 59.9 W	33 15 01.72 N 97 25 11.97 W
<b>2</b>	33 19 54 N 97 23 00.5 W	33 14 63.0 N 97 23 42.8 W	33 14 46.10 N 97 20 57.91 W	33 14 41.49 N 97 28 32.88 W
<b>3</b>	33 20 98.7 N 97 22 89.8 W	33 13 02.4 N 97 23 45.8 W	33 14 41.05 N 97 19 19.62 W	33 14 40.2 N 97 29 58.0 W
<b>4</b>	33 23 35.9 N 97 23 07.3 W	33 11 70.7 N 97 22 99.6 W	33 14 39.22 N 97 17 13.46 W	33 14 25.42 N 97 33 50.54 W
<b>5</b>	33 24 94.5 N 97 22 74.5 W	33 10 50.0 N 97 22 41.6 W	33 14 38.45 N 97 14 35.63 W	33 14 35.89 N 97 35 42.18 W
<b>6</b>	33 26 30.2 N 97 23 74.7 W	33 09 46.2 N 97 21 52.4 W	33 14 40.54 N 97 13 16.73 W	33 13 50.36 N 97 38 50.29 W
<b>7</b>	33 27 85.4 N 97 23 46.8 W	33 08 21.6 N 97 22 05.3 W	33 15 36.11 N 97 13 18.6 W	33 13 53.03 N 97 40 12.11 W
<b>8</b>	33 28 98.4 N 97 22 37.4 W	33 07 43.0 N 97 20 57.6 W	33 15 34.2 N 97 10 42.55 W	33 13 20.73 N 97 44 13.92 W
<b>9</b>	33 30 32.4 N 97 21 19.2 W	33 06 65.5 N 97 19 45.1 W	33 15 29.05 N 97 06 37.19 W	33 13 29.23 N 97 43 30.92 W
<b>10</b>	33 31 96.9 N 97 21 53 W	33 06 71.8 N 97 17 51.6 W	33 14 57.12 N 97 05 54.26 W	33 13 22.83 N 97 45 01.0 W

**Table 2.** GPS coordinates of each active red imported fire ant mounds where *P. curvatus* were released in Clarksville, Red River County, TX beginning on October 2007.

<b>Mound Number</b>	<b>Amount of RIFA Collected ( in grams)</b>	<b>IDENT Mound #</b>	<b>LATITUDE</b>	<b>LONGITUDE</b>
1	3.8	001	15SO317117	UTM3725686
2	4.8	002	15SO317128	UTM3725685
3	6.4	003	15SO317120	UTM3725669
4	3.1	004	15SO317146	UTM3725675
5	4.8	005	15SO317148	UTM3725671
6	6.1	006	15SO317136	UTM3725677
7	3.9	007	15SO317127	UTM3725673
8	1.1	008	15SO317157	UTM3725654
9	0.8	009	15SO317169	UTM3725671
10	4.7	010	15SO317158	UTM3725677
11	3.2	011	15SO317139	UTM3725674
12	3.5	012	15SO317141	UTM3725668
13	4.4	013	15SO317100	UTM3725716
14	--	014	15SO317099	UTM3725693
15	--	015	15SO317084	UTM3725703
16	--	016	15SO317077	UTM3725718
17	2.5	017	15SO317075	UTM3725722
18	7.4	018	15SO317076	UTM3725729
19	5.7	019	15SO317074	UTM3725734
20	2.0	020	15SO317057	UTM3725738
21	4.4	021	15SO317054	UTM3725756
22	4.3	022	15SO317049	UTM3725764
23	6.6	023	15SO317071	UTM3725733
24	1.6	024	15SO317039	UTM3725762

**Table 3.** Coordinates of each passive “pizza” trap placed in the release area at Ray Roberts Park, Denton County in order to detect the spread of *P. tricuspis* used on April 18 (traps 1-10) and November 1, 2007 (traps 1-15).

<b>Trap</b>	<b>Coordinates</b>
<b>1</b>	33 21.108 N 97 23 27.1 W
<b>2</b>	33 19 54 N 97 23 00.5 W
<b>3</b>	33 20 98.7 N 97 22 89.8 W
<b>4</b>	33 23 35.9 N 97 23 07.3 W
<b>5</b>	33 24 94.5 N 97 22 74.5 W
<b>6</b>	33 26 30.2 N 97 23 74.7 W
<b>7</b>	33 27 85.4 N 97 23 46.8 W
<b>8</b>	33 28 98.4 N 97 22 37.4 W
<b>9</b>	33 30 32.4 N 97 21 19.2 W
<b>10</b>	33 31 96.9 N 97 21 53 W
<b>11</b>	33 35 20.4 N 97 04 74.1 W
<b>12</b>	33 35 18.5 N 97 04 74.8 W
<b>13</b>	33 35 16.0 N 97 04 74.3 W
<b>14</b>	33 35 13.8 N 97 04 73.2 W
<b>15</b>	33 35 11.6 N 97 04 71.4 W

**Table 4.** Number of *P. curvatus* found on each passive “pizza” trap at each coordinate point from the original site at the M.T. Cole Ranch set out on April 18, 2007.

<b>Trap Number</b>	<b>North</b>	<b>South</b>	<b>East</b>	<b>West</b>
<b>1</b>	1	0	1	0
<b>2</b>	0	0	1	0
<b>3</b>	1	0	0	2
<b>4</b>	0	0	0	0
<b>5</b>	0	0	0	0
<b>6</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>8</b>	0	0	0	0
<b>9</b>	0	0	0	0
<b>10</b>	0	0	0	0



**Table 5.** Number of *P. curvatus* found on each passive “pizza” trap at each coordinate point from the original site at the M.T. Cole Ranch set out on November 1, 2007.

<b>Trap Number</b>	<b>North</b>	<b>South</b>	<b>East</b>	<b>West</b>
<b>1</b>	0	0	0	0
<b>2</b>	0	0	0	0
<b>3</b>	1	1	0	0
<b>4</b>	0	0	0	0
<b>5</b>	0	0	0	0
<b>6</b>	0	0	0	0
<b>7</b>	0	0	0	0
<b>8</b>	0	0	0	0
<b>9</b>	0	0	0	0
<b>10</b>	0	0	0	0

**Table 6.** Number of *P. tricuspis* found on each passive “pizza” trap found on 10 traps in Ray Roberts Park set out on April 18, 2007 and retrieved April 19, 2007.

<b>Trap Number</b>	<b>April 18<sup>th</sup></b>
<b>1</b>	0
<b>2</b>	0
<b>3</b>	0
<b>4</b>	0
<b>5</b>	2
<b>6</b>	0
<b>7</b>	0
<b>8</b>	0
<b>9</b>	0
<b>10</b>	1

**Table 7.** Number of *P. tricuspis* found on each passive “pizza” trap found on 15 traps in Ray Roberts Park set out on November 1, 2007 and retrieved November 2, 2007.

Trap Number	November 1 <sup>st</sup>
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	1
9	0
10	0
11	0
12	0
13	0
14	1
15	0

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