

# Final Report on the USDA-ARS Areawide Red Imported Fire Ant Management Project in Texas June 14, 2001 - May 31, 2006

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College Station, Texas May 31, 2007

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## Summary of research carried out

Two 300 acre sites of improved pastures infested with polygyne *Solenopsis invicta* were established in 2001. On each site, 50 permanent monitoring plots were established and monitored, 20 of these plots were located in the center of the pasture and 25 were located outside the perimeter for comparison of insecticide treated (inside) to untreated (outside) areas. Plots were monitored (both sites and all plots) for imported fire ant densities by sampling plots for: 1) imported fire ant mounds and foraging activity; 2) sampling arthropod density using pit-fall trap sampling; 3) monitoring for *Thelohania solenopsae* to document one site as free of this fire ant disease; and 4) treating both areas aerially using the “hopper blend” of Amdro®Pro (hydamethylnon) plus Extinguish® (methoprene) baits. Release and establish phorid fly species (*Pseudacteon tricuspis* in 2002 and *P. curvatus* in 2004) were conducted around the perimeter and monitor spread at one site (Five-Eagle Ranch). Plots were monitored semi-annually and re-treat as necessary based on an action level of 20 mounds per acre (35 to 40% “hits” on hot dog slices) to determine if presence of biological control agents or natural enemies increases the interval of needed insecticide applications. Protocol from 2001 was modified in 2005 to reduce sampling intensity but increase monitoring frequency of 300 acre plot sites.

## Introduction

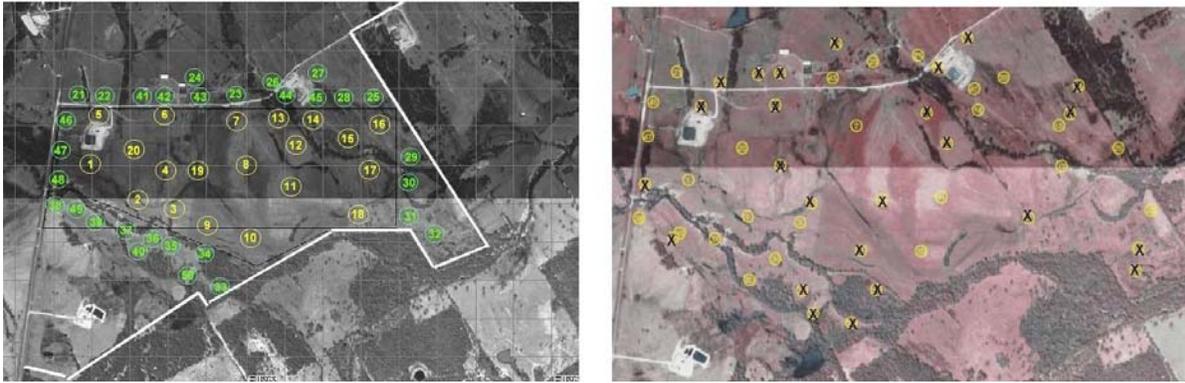
This report covers the time period of June 14, 2001 through May 18, 2007, for the Texas portion of the USDA Area-Wide Fire Ant Suppression Program initiated in 2001. In June, 2005 protocol changes were made to reduce sampling efforts at the two large area-wide sites established at the beginning of the program. Through August of 2005, spring evaluation had included all 100 plots at the original large improved pasture sites. Thereafter, plot numbers were reduced to 25 at each site (**Figs 1 and 2**). Sites were treated on May 31 and October 15 (2002), no treatments applied in 2003, April 15, 2004, May 17, 2005 and the last treatments on June 6, 2006.

## Methods & materials

### I. Field Sites

Five Eagle Ranch Glenn Rutherford, Manager 774-4511

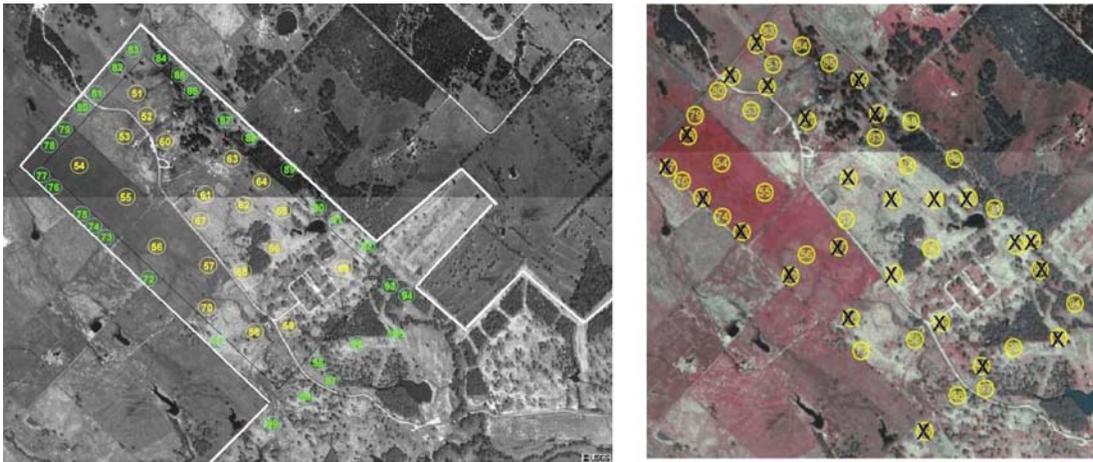
The “treatment” site, where biological control agents were released and established, is the Five Eagle Ranch (2,800 acres) located 5 mi north of Caldwell in Burleson County and owned by Mr. H. Norman Eagleton and managed by Mr. Glen Rutherford. The 300 acre treatment area was established in a wide, open, level creek bottom of mostly improved bermudagrass pastures.



**Figure 1.** Five Eagle Ranch plot map May 2002 (left), May 2005.

NK Cattle Company Marc Fratessa, Owner/Manager 589-2189

The “control” site, where no biological control agents were released, is the NK Cattle Company (1,800 acres) located 9 miles north of Bryan in along the Brazos-Robertson County line in far north Brazos County, Texas. It is owned by Marc and Diana Fratessa. The 300 acre treatment area was established near the front of the property on open level land with mostly improved bermudagrass pasture.



**Figure 2.** NK Cattle Company, “control” plot map, May 2002 (left) and May 2005.

## II. Monitoring and Treatment Efforts

Field plot establishment. Permanent plots (50 each) within the treatment area were established at both sites and both sites were mapped using GPS equipment and aerial photographs. An additional 50 plots were established around each treatment block to serve as untreated control monitoring plots. Weather data loggers were installed at both sites.

Mound counts. Mound counts and population index ratings were completed at both sites, data were collected through December 2005.

Food lure sampling. Recruitment sampling (Bar-S hot dog slices) was completed at both sites through June 2006. Food lure sampling using slices of Bar-S hot dogs exposed for 45-60 minutes were conducted during the spring and fall (2002-2004) and on four occasions in 2005 (May 26, July 5 & 6, September 9 & 13, and October 14 & 18. They were completed at both sites through June 2006.

Pitfall trapping. Pitfall trap sampling was completed in October 2005. Analysis of pitfall trap samples through Spring 2005 was completed and Fall 2005 pitfall trap analysis was completed by May 5, 2006. All collected and processed data were submitted to the in January 2006 to Dr. Roberto Pereira (USDA-ARS) for further analysis.

Bait treatments. Aerial application of hydramethylnon (AmdroPro®) plus s-methoprene (Extinguish®) bait was used for treating the sites. This “hopper blend” treatment was applied using USDA-APHIS aerial equipment at 1.5 lbs blended product per acre. Treatments were applied in May 31 and October 15 (2002), no treatments applied in 2003, April 15 (2004), May 17 (2005) and the last treatments on June 6 (2006).

### III. Biological Control Agent Establishment, Monitoring and Spread

Thelohania sampling. A PCR screening technique for *Thelohania*, developed by Dr. Forrest Mitchell of the Texas Agricultural Experiment Station in Stephenville, were used for detection and results were confirmed with microscopy. Both sites have had permanent plots sampled for *Thelohania* (10 mounds per plot). Both sites were initially screened for *Thelohania* infection, in 2002, of 20 1/8-acre circle plots within the treated area of Five Eagle Ranch, 4 out of 20 were found to harbor *Thelohania* infections, and of 30 plots outside the perimeter of the treated area 11 had infected ants. A few plots (2/20 in treatment area and 2/30 around perimeter) on the control site (NK Cattle Company) were also found to harbor *Thelohania* infected ants, and results were pending. Samples taken during fall 2002 showed *Thelohania* expansion on almost all the plots surrounding the treated areas for both sites. For year 2003, the infection fluctuated among the plots but remained relatively stable through the spring and summer and declining in the fall.

*Thelohania* samples for spring 2004 were collected prior to bait treatments so that mounds would be available in the treatment area. Forrest Mitchell has completed *Thelohania* analysis using PCR and microscopic techniques. A flaw in the published preparation procedure for PCR was detected and corrected. All samples since the beginning of the project were re-analyzed to corroborate PCR and microscopic findings. Few inconsistencies were found and those were corrected.

Phorid fly releases. The release of *Pseudacteon tricuspis* was completed at Five Eagle Ranch on May 13, 2002. At the treatment site (Five Eagle Ranch), 3,595 phorid flies were released between April 29 and May 11, 2002. Only 3 flies have were recovered by July 29, 2002.

An electric cattle prod was modified to aid in the detection of phorid flies. A replicated test conducted in September 2003 showed that electrically stimulated mounds had 100% fly observation while mechanically disturbed mounds had only 30% observation (Barr and Calixto 2005).

Robert Puckett developed a passive method of phorid fly collecting using Tanglefoot on a plastic tripod (April 10, 2006). The traps could be set out, left for some hours or until the next

day, then recovered and analyzed. This process appeared to be more efficient than disturbing mounds, though its accuracy compared to other detection methods had not been determined.

## Results

Mound counts. Densities of *S. invicta* remained significantly lower following the first bait application in treated plots compared to untreated in both demonstration sites (**Fig. 3**). Treatments provided roughly 90 percent control (range: <80 to 98% for sites in all participating states). Early in the program, the chemical treatment was been shown to be highly effective in most sites treated in Texas and other participating states.

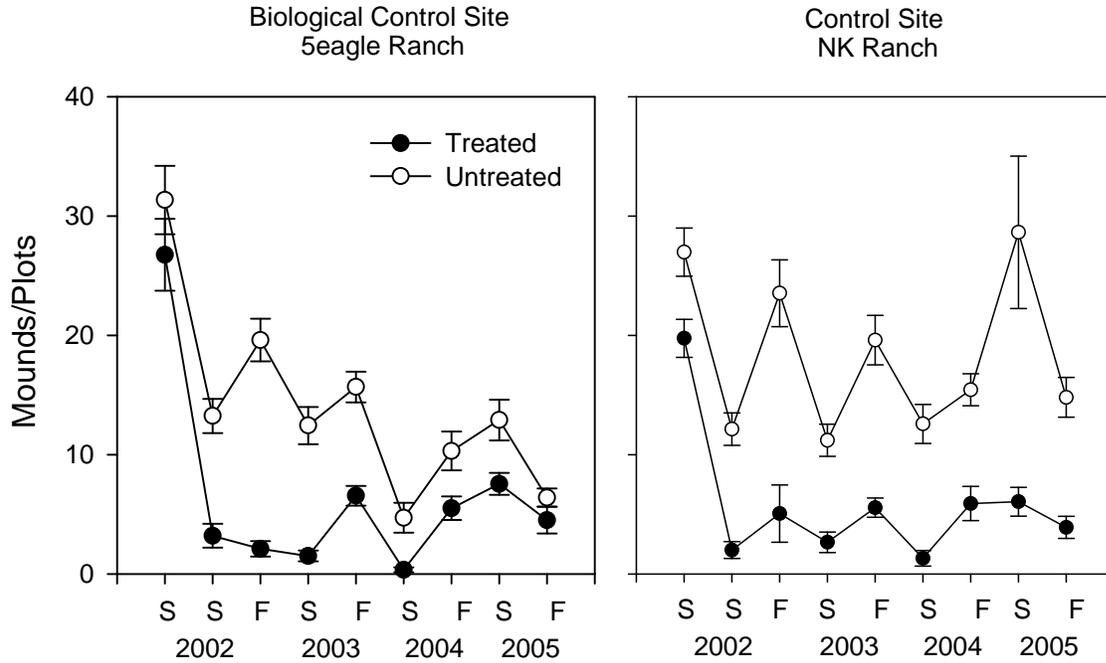
Location	Site	Size (acres)	Percent Control
Texas	“Control” site, NK Cattle Company	300	90% (10 wks)
	“Treatment” site, Five Eagle Ranch	300	88% (10 wks)

Spring evaluation in 2003 showed that fire ants had not reinfested plots above to threshold level, and no treatments were made in the spring. By fall, mound counts indicated that mound densities were well above the treatment threshold. Treatments were scheduled for November 3, but a combination of mediocre ant foraging and medical grounding of the pilot prevented bait application in fall, 2003. Both test sites were treated again with bait on April 15, 2004. Full evaluation routines (mound counts, pitfall trapping and food lure sampling) were conducted in the spring and fall of and spring of 2005. Both test sites were treated with bait on May 17, 2005. A full evaluation routine was conducted in the spring 2005. Both test sites were treated with bait on May 17, 2005. The reduced number of sample plots was evaluated in the fall of 2005.

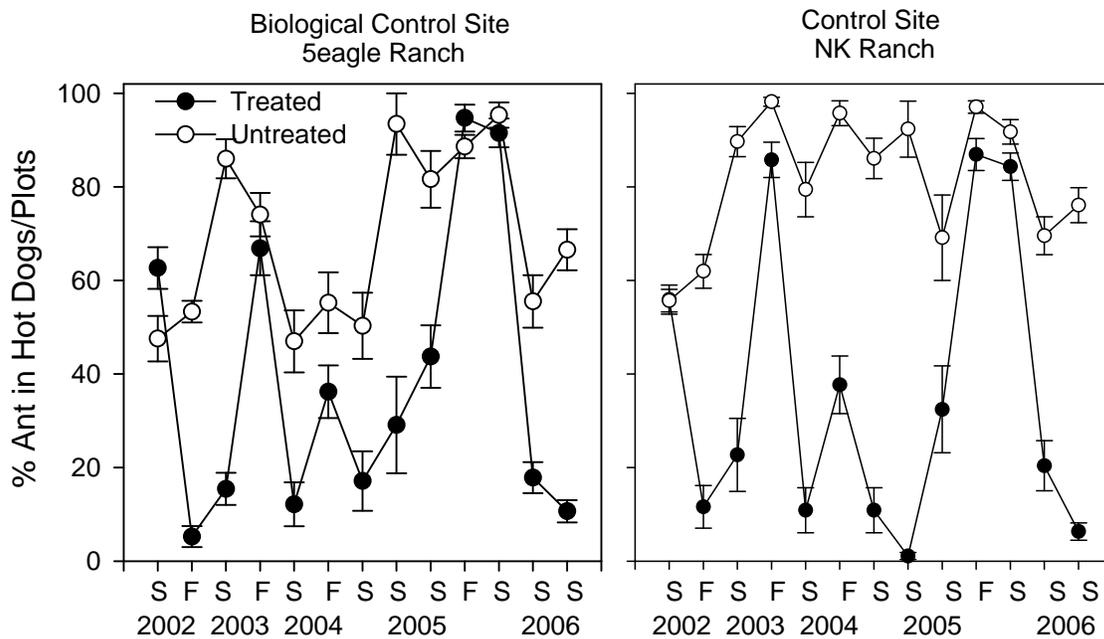
Food lure sampling. Foraging activity was reduced following bait treatment and remained lower in treated plots with recovery matching original infestation percentages in 2006 for both sites (**Fig. 4**). Average percent area of hot dog slices covered by foraging ants was lower in all plots treated aerially with the “hopper blend” of hydramethylnon plus methoprene ant bait products (Amdro®Plus and Extinguish®, respectively) on May 17, 2005 (also see **Fig. 4**).

In both sites, populations increased through the year, but at a lower level in insecticide treated sites. However, NK Cattle Company foraging ant activity was dramatically lower than at the Five Eagle Ranch, the “treatment site” where both species of phorid flies had been released and established. These results will make for difficult analysis when addressing the hypothesis addressing whether this combination of biological control agents is capable of reducing imported fire ant population level increases following chemical control application.

**Fig. 3.** Mound densities in both treated and untreated plots for the two demonstration sites for years 2002-2005. Arrows indicates the treatments.

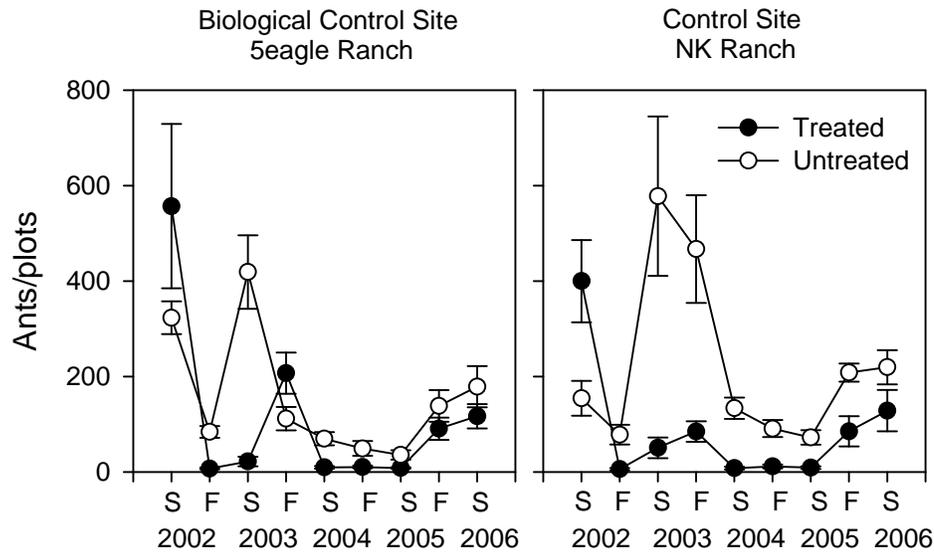


**Fig. 4.** Foraging of *S. invicta* on hot dogs recorded for treated and untreated plots for the two demonstration sites for years 2002-2006. Arrows indicates the treatments.



Pitfall sampling. Pitfall traps indicated a reduction of ant activity following bait application, and densities remained relatively lower in bait treated plots except for Fall 2005 for Five Eagle Ranch where numbers were temporarily higher in treated plots and thereafter remained similar for the rest of the evaluations. (**Fig. 5**) Results for pitfall trapping (April 25, 2005 at Five Eagle Ranch and April 29 at NK Cattle Company, **Fig. 5**) and mound counts and indexes (May 3, Dec. 2, 2005 at NK Cattle Company and May 5, Dec. 1, 2005 at Five Eagle Ranch, **Fig 3**) have been compiled for analysis.

**Fig. 5.** Densities of *S. invicta* in pitfall traps recorded for treated and untreated plots for the two demonstration sites for years 2002-2005. Arrows indicates the treatments.



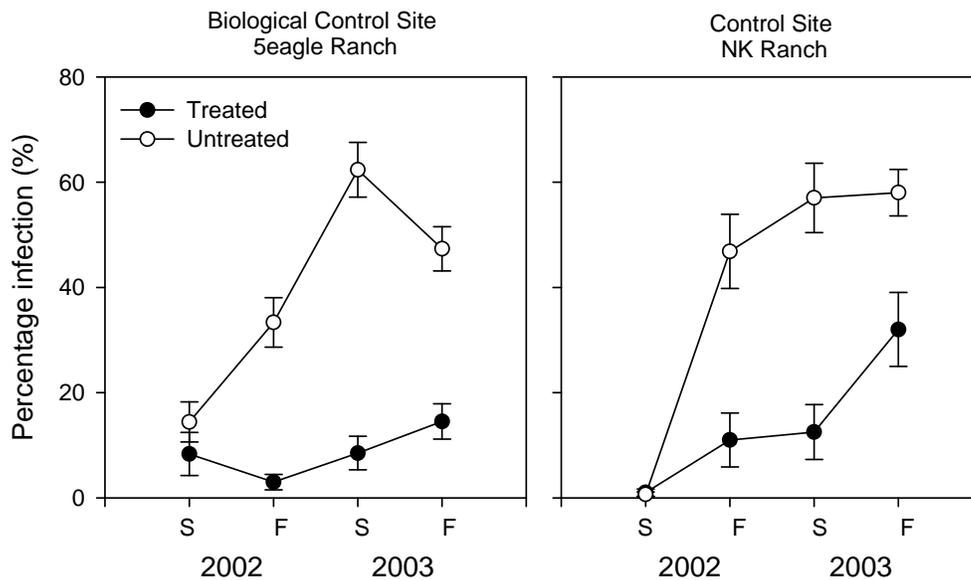
*Thelohania* sampling. A PCR screening technique for *Thelohania*, developed by Dr. Forrest Mitchell of the Texas Agricultural Experiment Station in Stephenville, was used for detection and results were confirmed with microscopy. Both sites have had permanent plots sampled for *Thelohania* (10 mounds per plot). Both sites were initially screened for *Thelohania* infection, in 2002, of 20 1/8-acre circle plots within the treated area of Five Eagle Ranch, 4 out of 20 were found to harbor *Thelohania* infections, and of 30 plots outside the perimeter of the treated area 11 had infected ants. A few plots (2/20 in treatment area and 2/30 around perimeter) on the control site (NK Cattle Company) were also found to harbor *Thelohania* infected ants, and results were pending. Samples taken during fall 2002 showed *Thelohania* expansion on almost all the plots surrounding the treated areas for both sites. For year 2003, the infection fluctuated among the plots but remained relatively stable through the spring and summer and declining in the fall (**Fig. 6**).

*Thelohania* samples for spring 2004 were collected prior to bait treatments so that mounds would be available in the treatment area. Forrest Mitchell has completed *Thelohania* analysis using PCR and microscopic techniques. A flaw in the published preparation procedure for PCR was detected and corrected. All samples since the beginning of the project were re-analyzed to corroborate PCR and microscopic findings. Few inconsistencies were found and those were

corrected.

By June 10, 2005, analysis of *Thelohania* samples indicated that the infection rate at he treatment (Five Eagle Ranch) site had suddenly dropped to pre-treatment levels. These results suggest that infection is highly cyclical. Due to budget concerns and difficulty in reliably detecting *Thelohania* through genetic testing, analysis of *Thelohania* samples are no longer conducted by Forrest Mitchell. Rather, analysis is currently conducted using microscopic methods. Samples collected during 2004- 2005 are being processed at this time by Robert Puckett and are expected to be completed by August 2007. At the present time (May 15, 2007), @95% of samples have been processed to microscope slides and @ 20% of these slides have been assessed for the presence of *Thelohania*. Fixed slides of these samples are being analyzed for a period of 2min. each. After which time if no *Thelohania* megaspores are observed the sample is scored as negative.

**Fig. 6.** Percentage ants infected with *Thelohania solenopsae*.



Phorid fly release. Beginning in the summer of 2003, substantial numbers of *P. tricuspidis* phorid flies were observed in the release area of Five Eagle Ranch. On November 8, 2003, flies were detected out to a maximum range of 3 km from the release site and out at least 1 km in all directions. It was common to observe five to ten flies over a mound within five minutes of electrical disturbance in the release area throughout the fall. Furthermore, a comparison of pre-release and Fall 2004 mound counts provide strong correlations of increasing phorid fly numbers and decreasing fire ant mound numbers in one area of Five Eagle Ranch.

On March 31, 2004, phorid flies were detected on numerous mounds in the release area of Five Eagle Ranch, though in small numbers with long detection times relative to last fall. By May 2004, observations indicated rapidly increasing numbers of *P. tricuspidis*.

To summarize, this area (10 plots) had about 40% more mounds than the remaining plots prior to phorid release. By fall 2004, mound counts were almost identical in all areas. By November 2004, flies were detected out to a maximum range of 10 km from the release site and

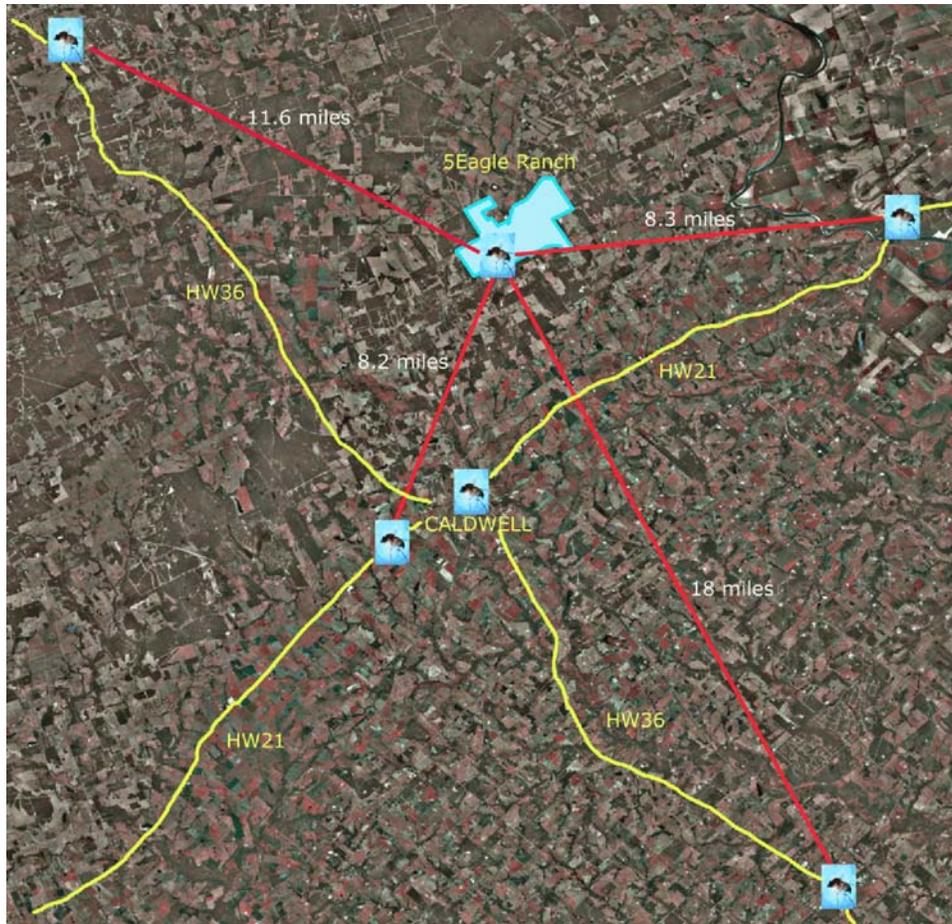
out at least 3 km in all directions. It was common to observe five to ten flies over a mound within five minutes of electrical disturbance in the release area throughout the fall. In May 2004, one release of *P. curvatus* had been completed and ants were being collected for a second sequential release. The following year, a single specimen was recovered near the release site at Five Eagle Ranch in May 2005, indication establishment. This species was detected in large number in the fall of 2005. Expansion was documented to have spread several hundred yards from the release site, particularly to the Northeast.

*Pseudacteon tricuspis* flies had expanded at least 5 miles to the south by September 2006, from previously reported expansion (**Fig. 7**) and *P. curvatus* was radiating several hundred yards from the initial release site. PTS Traps (**Fig. 8**) developed by Robert Puckett were used on September 26, 2006 to census Phorid fly expansion from the initial release site at 5-Eagle Ranch. Traps were placed to the east and west at 1 mile increments along 15 mile transects beginning at the intersection of FM 2000 and HWY 21 just east of Caldwell, TX. Phorids were detected 11 miles east (Brazos, Co.) and 5 miles west (**Fig. 7**). Northern and southern transects originated at the intersection of Country Club Rd & FM 36 and HWY 21 & FM 36 respectively. Again, traps were placed at 1 mile increments along 15 mile transect. Flies were detected at the 15<sup>th</sup> trap to north in the town of Milano, TX (Milam, Co) and at 14<sup>th</sup> trap to the south in the town of Lyons, TX (Burleson, Co.). High numbers of flies on traps at the end of the north and south transects indicate that we have not yet established these expansion boundaries and this will be addressed during October 2006.

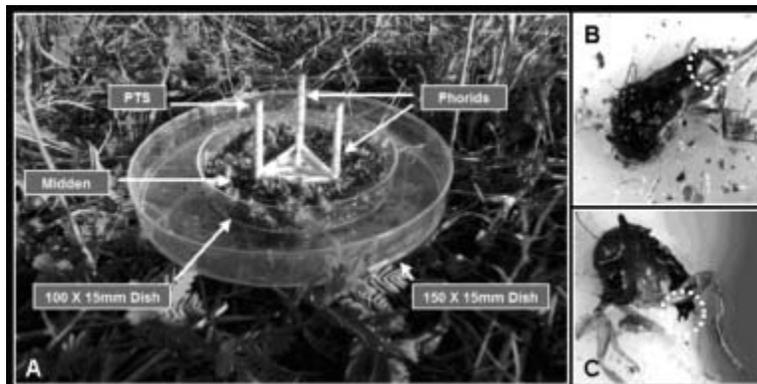
## Impact

This Area-Wide Suppression of Imported Fire Ant project demonstrated the integration of biological and chemical fire ant control in improved pastures in central Texas. Results will benefit the people of Texas provided educational programs conducted by Texas Cooperative Extension personnel wherein they are presented as examples of practices available to achieve control using these Integrated Pest Management tactics. This demonstration documented results of aerial application of improved pastures using the "hopper blend" treatment (consisting of the mixture of hydramethylnon and methoprene fire ant bait products, AmdroPro® plus Extinguish®) at a cost of roughly \$17/acre (including cost of application) to provide and maintain suppression of imported fire ants at about 80%. This practice can be effectively implemented where such a cost of treatment is justified. The natural enemies of imported fire ants, *Pseudacteon* species of phorid flies and the microsporidian *Thelohania sp.* have been established and spread from the release site. Although impact from these natural enemies has not been determined, the effects of these agents are expected to persist and aid in possibly providing some level of sustainable suppression of imported fire ants.

**Fig. 7.** *Pseudacteon tricuspis* expansion, Nov. 2003.



**Fig. 8.** Passive trap for monitoring *Pseudacteon* spp.



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Barr, C. L., and A. Calixto. 2005. Electrical stimulation of *Solenopsis invicta* to enhance phorid fly, *Pseudacteon tricuspis*, detection. *Southwestern Entomol.* 30:165-168.

## Citation of periodicals reporting work done under the project and other relevant literature references

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- Calixto, A., A. Dean and M. K. Harris. 2007. (*In Press*). Sampling ants with pitfall traps: propylene glycol vs water as kill/preservative agent. (*Southwest. Entomol.*)
- Calixto, A., A. Dean, A. Knutson and M. K. Harris. 2006. Density Changes of Two Earwigs, *Labidura riparia* (Pallas) And *Euborellia annulipes* (Lucas) Following Fire Ant Reduction in Mumford, Texas. *Southwest. Entomol.* 31: 97-101.
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### Extension publications

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[http://fireant.tamu.edu/materials/newsletters/fa\\_trails/2002/pdf/FATRAILS6\\_002\\_usda.pdf](http://fireant.tamu.edu/materials/newsletters/fa_trails/2002/pdf/FATRAILS6_002_usda.pdf)

### Media

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- 2) "A fire ant's worst nightmare". *The Dallas Morning News* - Article, Interview to Alejandro Calixto and Elizabeth Hickman, October 25, 2002
- 3) "Management of fire ants using multiple techniques" - Interview to Dr Charles Barr, *AgNews*, Texas A&M University, October 8, 2003
- 4) "Phorid fly: fire ant's predator" - *AgNet Radio*, Texas A&M University, Radio interview to Dr Charles Barr -
- 5) "Unwanted guests". *Ft. Worth Telegram* - News article, October 20, 2003. Interview to Dr Charles Barr and Alejandro Calixto.
- 6) "Phorid flies possible fire ant control" - *Southwest Farm Press*, November 20, 2003. Interview to Dr Charles Barr.
- 7) "In Texas, fire ants are a force to be reckoned with deadly, destructive insects prove to be difficult to eradicate" - *The Washington Post*, April 18, 2005. Interview to Dr Charles Barr.
- 8) "New fire ant control is given thumbs up" - *The Eagle*, July 6, 2005. Interview to Dr Charles Barr

- 9) "Fire ants had better head to lower ground" - San Antonio Express News, December 30, 2005. Interview to Molly Keck and Alejandro Calixto.
- 10) Barbee, B. 2002. USDA-ARS Area-wide Suppression of Fire Ants Project with Frequently Asked Questions, Agricultural Communications news releases, June 10.
- 11) "Texas A&M Doctoral Student Builds Better Fly Trap" - Interview with Robert Puckett, AgNews, Texas A&M University, December 6, 2006.