

# Combining Biological and Chemical Controls for the Management of Red Imported Fire Ants (Hymenoptera: Formicidae)

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**ABSTRACT:** Two South American natural enemies of imported fire ants were first detected or released in the United States approximately 10 years ago. The fire ant pathogen, *Thelophania solenopsae* Knell, Allen, and Hazard, was found in the U.S. in 1996 and a parasitic phorid fly from Brazil, *Pseudacteon tricuspis* Borgmeier, was released in 1997 and both are well established in fire ant infested areas. As biological control agents of the red imported fire ant, *Solenopsis invicta* Buren, their colony-level impact in the field is often indirect and subtle as they work slowly by debilitating queens and impeding foraging by workers. Their effect on colony densities may be inadequate in sensitive sites, where people have a low tolerance for fire ant stings, and where control may require faster-acting insecticide treatments. Comparisons of *S. invicta* populations and the presence of other ants were made among field sites that 1) were treated with insecticide containing fipronil and where the biocontrol agents *T. solenopsae* and *P. tricuspis* were also released and established (integrated site); 2) were treated only with the fipronil insecticide (chemical site); and, 3) were not treated (untreated site). *S. invicta* populations were suppressed by  $\geq 95\%$  for 3 years at the integrated site. In the chemical site, *S. invicta* control was  $\leq 85\%$  after 1.4 years, while the untreated site had an average 32% increase in population. Average prevalence of *T. solenopsae* among nests per plot peaked at 72% and *P. tricuspis* was observed at the release site and up to 5 km away. The average percentage of ants other than *S. invicta* collected in pitfall traps in the insecticide-treated area of the integrated site increased from 13% before treatment to 70% for the last 2 years of the study. In the chemical site, the percentage of non-*S. invicta* ants was 0.4% before insecticide application and averaged 9% for the final 2 years. Non-*S. invicta* ants averaged 9.6% (range, 2.7-17.3%) in the untreated site for the entire study. The extended reduction in *S. invicta* populations in the integrated site demonstrated a potential impact of the establishment of biological control agents for imported fire ants.

Since its inadvertent introduction into the United States in the early 1930s, the aggressively stinging red imported fire ant, *Solenopsis invicta* Buren, continues to expand its range, and it currently occupies about 134.8 million ha [333 million acres] (Callcott and Collins 1996, Tschinkel 2006, APHIS 2006). This invasive pest has also begun to travel further abroad, where within the last seven years, infestations have been found in Australia, New Zealand, Taiwan, Hong Kong, mainland China, and Mexico. In the U.S., attempts at fire ant eradication and regional control in the southern infested states occurred from 1957 through 1977 (Canter 1981). Utilization of contact insecticides such as dieldrin and heptachlor, and later bait containing mirex, were highly controversial and ultimately unsuccessful (Lofgren 1986, Tschinkel 2006). The more recent (2000-2003) eradication program in California used baits containing insect growth regulators and metabolic inhibitors,

as well as contact insecticides, but the state program ended when funding was discontinued (Klotz et al. 2003, Orange County Vector Control District, <http://www.ocvcd.org/fireants/default.htm>). Eradication of long-established fire ant infestations in the U.S. is no longer considered feasible, and control programs focus on site-specific application of fire ant baits and/or contact insecticides (Drees and Gold 2003).

Interest in natural enemies of fire ants from South America as potential biological control agents has been ongoing since the 1970's (Williams et al. 2003). In 1997, the first successful releases of the fire ant decapitating phorid fly, *Pseudacteon tricuspis* Borgmeier, were made in Florida, and the fly has subsequently established and spread (Porter et al. 2004, Pereira and Porter 2006). Direct mortality caused by the flies developing within individual ants is estimated to be <1% (Morrison and Porter 2005a). A greater impact of the

flies occurs by the disruption of fire ant foraging behavior. When *P. tricuspidis* is present, fire ants display behaviors that reduce their exposure to attacking flies, and the fact that fire ants have evolved such defensive mechanisms against the flies suggests colony level impacts (Porter 1998, Wuellner et al. 2002). Effects of foraging disruption by *P. tricuspidis* under laboratory conditions have been demonstrated (Mehdiabadi and Gilbert. 2002, Mottern et al. 2004), but colony impacts in the field were found to be less than the estimated 15-30% resolution of the study design (Morrison and Porter 2005b). Nevertheless, all the cited studies above cautioned that only a single fly species was tested, out of the nearly 20 fire ant decapitating *Pseudacteon* phorids known in South America (Porter and Pesquero 2001), and that more time and species may be needed to detect population-level effects.

In addition to the decapitating phorid flies, a microsporidian pathogen of fire ants from South America, *Thelohania solenopsae* Knell, Allen, and Hazard, was found in the U.S. in 1996 (Williams et al. 1998, 2003). This pathogen infects and debilitates fire ant queens, resulting in reduced oviposition and premature death (Williams et al. 1999). Field studies in Argentina and the U.S. documented smaller fire ant colonies or populations in infected sites (Briano et al. 1995a, 1995b, Cook 2002, Oi and Williams 2002, Fuxa et al. 2005). Because *T. solenopsae* causes a slow colony decline, reductions in field populations are moderated by concurrent re-infestations (Oi and Williams 2002) and the level of control is inadequate for sensitive areas where the risk of stings is a major and imminent concern.

While these biological control agents of fire ants currently do not achieve the level and speed of control afforded by insecticides, they are established in the U.S. and are self-sustaining. Their impact may be realized more quickly when they are combined with chemical control methods. Managed landscapes, where land managers have a low tolerance for fire ants, often are treated with insecticides, with surrounding unmanaged areas serving as a source for fire ant reinvasions. In the following field study, we examined the potential benefits of integrating biological and chemical controls for fire ants.

## Methods

The study was conducted at three sites in Richland County, South Carolina, from June 2000 to May 2003. It consisted of 1) an integrated control site (integrated site) where two biological control agents were released and an insecticide was applied; 2) a chemical control site, (chemical site) where only an insecticide was applied; and 3) an untreated site. The latter two sites were both situated at the Fort Jackson U.S. Army Training Center, while the integrated site was located at the South Carolina Air National Guard, McEntire Joint National Guard Station. Because of the dispersal potential of the biological control agents, each site was located 13 to 18.5 km (8-11.5 miles) apart. For each site, 12 or 16 circular plots (0.05 ha/plot = 1/8 acre/plot) were established to monitor ant populations. The integrated and chemical sites each contained a 1.8 ha (4.5 acre) area that was treated with insecticide and each treated area encompassed eight of the circular monitoring plots. On the periphery of the insecticide-treated areas, eight and four circular plots served as internal controls at the integrated and chemical sites, respectively. In the untreated site, 12 circular plots were monitored. Plots not treated with insecticide and without releases of biological control agents are hereafter referred to as "controls." Thus, due to the need for each control strategy site to be widely separated from the other sites, the study consisted of a single experiment unit with multiple sampling plots.

A granular contact insecticide containing 0.1% fipronil (Chipco Choice, Aventis Environmental Science) was applied on June 9, 2000 at an application rate of 14 kg/ha (12.5 lbs/acre). The

application was made with a positive displacement applicator (Gandy Co., Owatonna, MN) pulled by an all-terrain vehicle, and was the only insecticide treatment made during the 3-year study period. At the integrated site, two fire-ant-specific biological control agents were also released in peripheral locations near the insecticide-treated area.

**Biological Controls.** In eight circular plots bordering the insecticide-treated area at the integrated site, *T. solenopsae* was introduced into 41 and 22 nests on June 8 and November 1, 2000, respectively. Inoculations were made by pouring 3-4 g of live brood from *T. solenopsae*-infected colonies into a small opening made in each nest. Brood was separated from adult ants before inoculations by pouring groups of brood and adults between card stock paper or file folders. Adult ants would grasp the paper or folder, while brood would roll off without the adults onto another folder, thus isolating the brood after several pours. Brood used for inocula had infection rates of 81 and 70% for the June and November inoculations, respectively. Infection rates were based on the examination of 10 individual slide mounts of prepupae or 4<sup>th</sup> instar larvae per infected colony used as a source of inocula (Oi and Williams 2002). The presence of *T. solenopsae* in the field was determined by phase microscopy of wet mounts prepared from macerated adult worker caste ants (ca. 30-50 ants) collected from all active nests (Oi and Williams 2002, Oi et al. 2004) in the integrated site plots, and from 10 active nests per plot at the chemical and untreated sites. Adult ant samples for *T. solenopsae* determinations were collected at the same time fire ant population assessments were made.

The other biological control agent released at the integrated site was the decapitating phorid fly parasitoid of fire ants, *P. tricuspidis*. About 3,000 flies were released approximately 1 km from the plots over a 2-week period (June 14-30, 2000). Every afternoon, 30-60 adult flies were released at each of 5-10 fire ant nests that were disturbed every few minutes for 2 h to expose adult ants to ovipositing flies. Establishment of flies was determined 2-3 times a week beginning 35-40 days after the initial release. After several weeks, inspections were reduced to once or twice a month until the flies were consistently observed and clearly established. Subsequently, fly dispersal was monitored in the spring and fall, when fire ant populations were assessed, by observing and collecting flies at disturbed nests located at approximately 0.8 km intervals away from the release site in several directions. Locations where flies were present and absent were recorded using a global positioning system.

**Fire Ant Populations.** Fire ant populations were determined within each circular plot by counting active fire ant nests and estimating the colony size and reproductive status of each nest by using the USDA population index (PI) rating system (Lofgren and Williams 1982). In this system, nests were opened with a small-bladed (15.2 W x 21.6 L cm) shovel and the number of adult ants visually categorized among five ranges (1-100; 101-1000; 1001-10,000; 10,001-50,000; and >50,000) and the presence or absence of worker-caste larvae and pupae determined. The absence of worker-caste immature stages and/or only the presence of reproductive-caste immature stages indicated that the colony was declining and resulted in a lower PI rating. Fire ant population assessments were made in the spring and fall seasons, 1-2 days prior to the insecticide treatment (June 2000), and at 16 (Sep 2000), 48 (May 2001), 72 (Oct 2001), 101 (May 2002), 119 (Sep 2002), and 153 (May 2003) weeks after treatment.

Because the control strategies could not be replicated within individual sites, the circular plots were subsamples of each area with or without the insecticide application for each site. Fire ant population changes for each sampling date were reported as average percentage reductions of the PIs obtained before chemi-

cal application or biological control releases. Confidence intervals (95%) were also calculated for each average reduction. Averages with non-overlapping confidence intervals were considered to be significantly different.

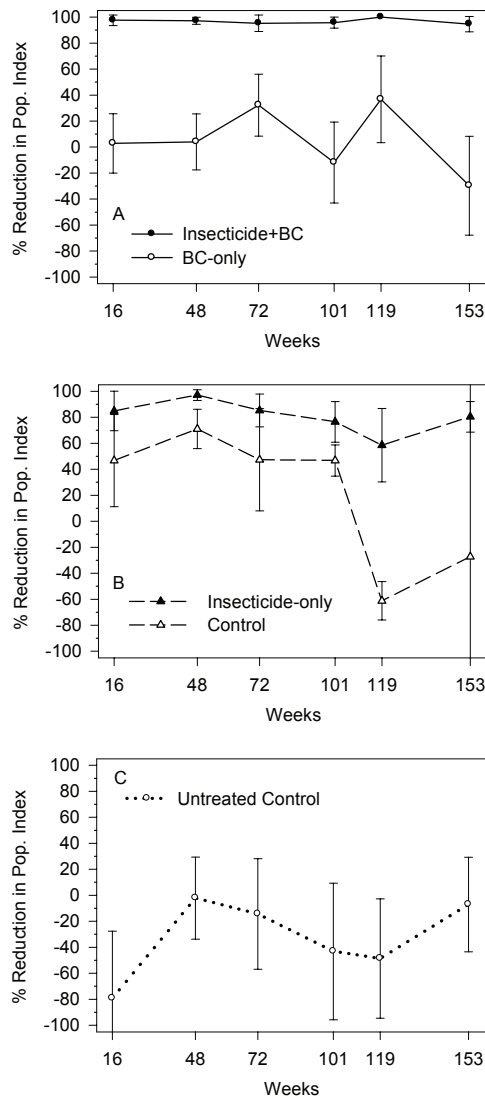
Pearson's correlation (SAS Institute 2002) was used to examine the association between percent infection per plot and the sum of the PIs per plot over all sample dates from the inoculated plots within the integrated site. A paired *t*-test (SAS Institute 2002) compared the sum of the PIs before inoculation and at week 119, when average *T. solenopsae* infection rate per inoculated plot was highest.

To provide spatial documentation of infected and uninfected colonies, all active nest locations were mapped using geographical information systems (GIS). A global positioning system (GPS) unit with 3 - 5 m accuracy was used to locate the sample plot centers. During fire ant population assessments, each nest was marked with a survey flag labeled with an identifying number. A survey team visually georeferenced the location of each nest on a handheld computer running GIS software. To visually georeference nests, flags were placed on the four directional cardinal points (north, south, east, and west). One individual then stood in the center of the circle and another visited each marked nest, and together they estimated the azimuth and distance from the center of the circle. Using the map loaded on the handheld computer, the cardinal points, and the circles, nest locations were digitally added to the map. This provided an accurate representation of the fire ant nest distribution and was superior to using the GPS, which did not have the submeter accuracy necessary for mapping individual nests.

**Pitfall Trapping.** Ant diversity among the study sites was assessed by pitfall traps. Traps were uncapped, polystyrene plastic snap-cap vials (9 dram [33.3 ml], 25 mm diameter x 70 mm height) filled 1/3 full of automotive anti-freeze solution (propylene glycol). Vials were placed into the ground such that the lip of each vial was flush with the ground surface. A total of eight traps were installed per circular plot, and were positioned at the four cardinal directional points 3.7 (12 ft) and 7.3 (24 ft) m from the plot center. This was approximately 9.1 and 5.5 m from the edge of the plot, which had a radius of 12.8 m (42 ft). Traps were set on dates when fire ant nest populations were monitored and were collected approximately 48 h later. Ants in the pitfall samples were counted and identified to genus in the laboratory. The number of genera and ant abundance per plot were tabulated for each site. Average percentages and 95% confidence intervals of *S. invicta* and non-*S. invicta* ants trapped per plot for each sampling date were reported for each treated and control area among the sites.

## Results and Discussion

**Fire Ant Populations.** Average percent reductions in PIs of the insecticide-treated and control plots among the integrated, chemical, and untreated sites are shown in Fig. 1. Fire ant population reductions in the insecticide-treated plots at the integrated site (insecticide+BC) were  $\geq 95\%$  for the duration of the study. In the surrounding area where biological controls were introduced (BC-only), fire ant populations fluctuated between a 37% reduction to a 30% increase from initial populations (Fig 1a). The lack of overlapping 95% confidence intervals indicated that the insecticide+BC plots had significant reductions in fire ant populations relative to the BC-only areas, as well as the untreated site (Fig. 1c). In the chemical site, insecticide-treated plots (insecticide-only) had reductions of  $\geq 85\%$  through week 72. Thereafter, PI reductions fluctuated between 58 and 80%, indicating that fire ants were beginning to re-infest the insecticide-only area. In the control plots on the periphery of the insecticide-only area, fire ant populations were quite variable among sampling dates, ranging from a 71% decrease to a 61% increase (Fig. 1b). The higher variability in the control may be partially attributed to the lower number of plots, portions of which, on some dates, had



**Fig. 1.** Reductions in *S. invicta* population indices per plot (avg. % reduction  $\pm$  95% CI) after insecticide application and biological control releases among areas within the a) integrated site, where fire ant biological controls were released and insecticide was applied; the b) chemical site, which was treated with insecticide only; and the c) untreated site, where neither biological or chemical controls were used. Filled and open symbols represent plots with and without insecticide treatment, respectively. Negative reductions represent increases in *S. invicta* populations relative to pretreatment population estimates. Weeks after insecticide application or biocontrol releases are indicated on the x-axis.

more shading due to seasonal growth of vegetation, which created a suboptimal fire ant habitat. Fire ant populations at the untreated site averaged 32.4% ( $\pm 29.8$  SD) higher than the initial population during the study (Fig. 1c).

**Biological Controls.** The percentages of *T. solenopsae*-infected nests per plot among all sites are presented in Table 1. At the integrated site, *T. solenopsae* became pervasive, increasing from 0 to a maximum of 72% in Sept. 2002 (week 119). *T. solenopsae* spread to non-inoculated plots in the insecticide+BC area as fire ants began to re-infest some of the plots (Fig. 2). In the chemical site, *T. solenopsae* was first detected in Oct. 2001 (week 72) and a maximum of 35 (22%) nests were infected at week 119. The occurrence of *T. solenopsae* in a non-inoculated site may have been the result of a natural spread or possibly contamination introduced by study participants. The latter seemed unlikely, given the effort required

**Table 1. Percentage of *T. solenopsae*-infected fire ant nests per plot for sites where fire ant biological controls were released and insecticide applied (Integrated site), treated with insecticide only (Chemical site), and where biological or chemical controls were not used (Untreated site), Richland County, South Carolina. A total of 41 and 22 nests were inoculated on 8 Jun 2000 and 1 Nov 2000, respectively, in the Biocontrol-only plots.**

Week	Avg. $\pm$ SEM % infected nests/plot (No. nests infected/ no. examined <sup>d</sup> )				
	Integrated Site		Chemical Site		Untreated Site
	BC <sup>c</sup> -only (n=8) <sup>b</sup>	Insecticide+BC (n=8)	Control (n=4)	Insecticide (n=8)	Control (n=12)
0	0.0 $\pm$ 0.0 (0/231)	0.0 $\pm$ 0.0 (0/108)	0.0 $\pm$ 0.0 (0/37)	0.0 $\pm$ 0.0 (0/80)	0.0 (0/108)
16	16.8 $\pm$ 5.2 (24/193)	-- <sup>d</sup>	-- <sup>d</sup>	-- <sup>d</sup>	-- <sup>d</sup>
48	22.0 $\pm$ 5.0 (40/224)	0.0 $\pm$ 0.0 (0/26)	0.0 $\pm$ 0.0 (0/12)	0.0 $\pm$ 0.0 (0/9)	0.0 (0/98)
72	36.8 $\pm$ 4.9 (47/130)	12.9 $\pm$ 9.7 (2/13)	0.0 $\pm$ 0.0 (0/26)	20.2 $\pm$ 12.4 (4/24)	0.0 (0/99)
101	30.0 $\pm$ 6.1 (65/211)	18.3 $\pm$ 13.0 (3/11)	8.3 $\pm$ 8.3 (3/22)	1.3 $\pm$ 1.3 (1/42)	0.0 (0/96)
119	72.4 $\pm$ 7.1 (138/175)	-- <sup>e</sup>	22.9 $\pm$ 7.6 (24/87)	11.0 $\pm$ 5.8 (11/72)	0.0 (0/99)
153	49.6 $\pm$ 8.1 (134/260)	20.0 $\pm$ 13.3 (5/12)	31.1 $\pm$ 19.7 (17/61)	8.3 $\pm$ 5.5 (3/31)	0.0 (0/93)

<sup>a</sup> Total number of infected nests / no. nests examined for *T. solenopsae* over all plots within a site-treatment location.

<sup>b</sup> Number of plots per site-treatment location

<sup>c</sup> BC, Biocontrol

<sup>d</sup> Nests not examined

<sup>e</sup> Nests not present

to initiate infections artificially. In the untreated site, *T. solenopsae* was not detected during the study.

In the BC-only plots at the integrated site, percentages of *T. solenopsae* infection per plot were not correlated to PI sums ( $r = -0.051$ ,  $P = 0.71$ ,  $n = 55$ ). However, the PI sums per plot when *T. solenopsae* infection was highest (week 119) were significantly lower than PIs before inoculation (week 0) ( $t = 2.379$ ,  $df = 14$ ,  $P = 0.049$ ). Average PI sum per plot on week 0 was 429 ( $\pm 184$  SD) and declined 28% to 311 ( $\pm 218$  SD) on week 119. These results were similar to those reported by Oi and Williams (2002) where the lack of correlation was attributed to asynchronous onset of new infections, which increase infection rates, while declines in PIs were not yet apparent due to a time lag before the disappearance of brood. A comparable 33% decline in PIs was also reported by the same authors between sampling dates with low and high *T. solenopsae* infections.

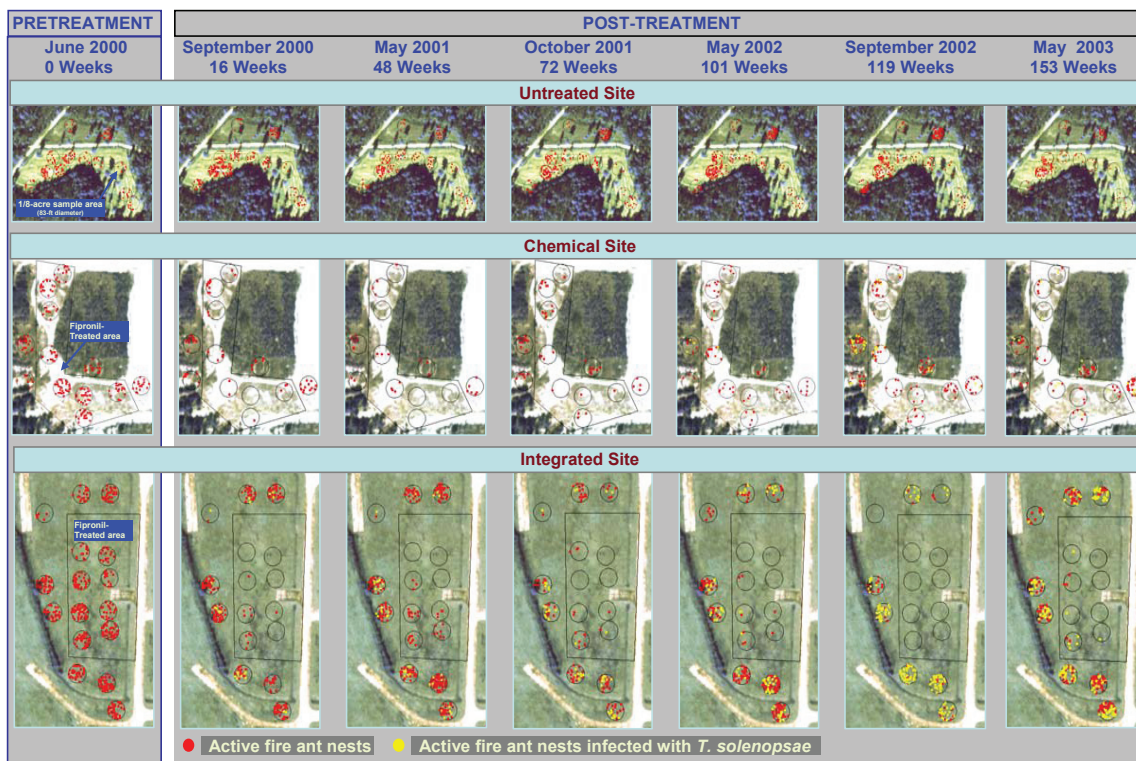
Phorid flies were first observed attacking fire ants at three nests at the release site 16 weeks after their initial release. Flies were observed at this site the following spring (week 48), and thus successfully overwintered. At 72 weeks, 28 flies were seen at 11 fire ant colonies within 20 minutes. This intensity of flies attacking fire ants indicated that the fly population had increased. In subsequent surveys, phorid flies were consistently observed at the release site and up to 5 km (3 miles) away, providing further evidence of their establishment and spread.

**Pitfall trapping.** Percentages of *S. invicta* and non-*S. invicta* ants collected in pitfall traps varied among the sites, especially within the treated plots. In the plots treated with insecticide in the integrated site, the percentage of non-*S. invicta* ants increased from 13% before treatment was applied to 50% by the 48<sup>th</sup> week and averaged 70  $\pm$ 6% ( $\pm$ SD) thereafter. Percentages of *S. invicta* had a reciprocal decrease

(Fig. 3a). In the treated plots in the chemical site, the percentages of non-*S. invicta* ants increased at lower rates during the study, accounting for 0.4% of trapped ants before treatment and averaging 9  $\pm$  5% ( $\pm$ SD) after week 48 (Fig. 3c). Average non-*S. invicta* percentages for plots not treated with insecticide at all three sites were less than 17% on all sampling dates (Fig. 3b, 3d, 3e). Confidence intervals of 95% did not overlap between percentages of *S. invicta* and non-*S. invicta* ants within all plots and sampling dates except for the insecticide+BC plots in the integrated site (Fig. 3). Thus, *S. invicta* was significantly more prevalent, percentage-wise, in pitfalls than the other ants in the area without the combination of biological and chemical controls.

The abundance of *S. invicta* in pitfalls decreased 97 and 95% 16 weeks after insecticide was applied to the integrated and chemical sites, respectively. Average number of *S. invicta* per plot remained below 39, or 2%, of the pre-treatment counts from weeks 48 to 153 in the insecticide+BC, integrated site plots. In the insecticide-only area of the chemical site for the same sampling dates, excluding week 72, or 16% of the initial trap counts (Table 2). In the BC-only plots and in the control plots of the chemical site, *S. invicta* counts were less than the average number of *S. invicta* per plot trapped initially in June 2000, with decreases ranging between 10 and 68%, and 8 and 43%, respectively. In the untreated site, *S. invicta* ranged from 58 to 176% of the initial collection (Table 2).

Abundance of non-*S. invicta* ants in the insecticide+BC plots of the integrated site initially decreased 93% from the initial count 16 weeks after treatment; on week 101 increased to 16% higher than the initial count; and then had a 76% decrease on the final sampling. The fluctuations in non-*S. invicta* abundance were initially similar



**Fig. 2.** Spatial distribution of active *S. invicta* nests within 0.05 ha circular plots located in the untreated, chemical, and integrated sites. Areas within the polygon and rectangle delimited by black solid lines in the chemical and integrated sites, respectively, were treated once with a granular, contact insecticide formulation of fipronil. Red and yellow dots denote uninfected and *T. solenopsae*-infected *S. invicta* nests, respectively.

to that of the BC-only plots where reductions from pre-treatment counts were 94, -15, and 98% for the same sampling periods. Average number of trapped non-*S. invicta* ants per insecticide+BC plot ranged from 3 to 50, while in the BC-only plots, averages ranged from 0.4 to 21 (Table 2). In the insecticide-only and control plots of the chemical-site, non-*S. invicta* abundance was low with an average of less than 4 ants being trapped per plot, with the exception of treated plots on week 101 where 14 ants were collected. Non-*S. invicta* abundance in the untreated site ranged between 6 and 22 ants per plot, excluding the rain-soaked week 119 (Table 2).

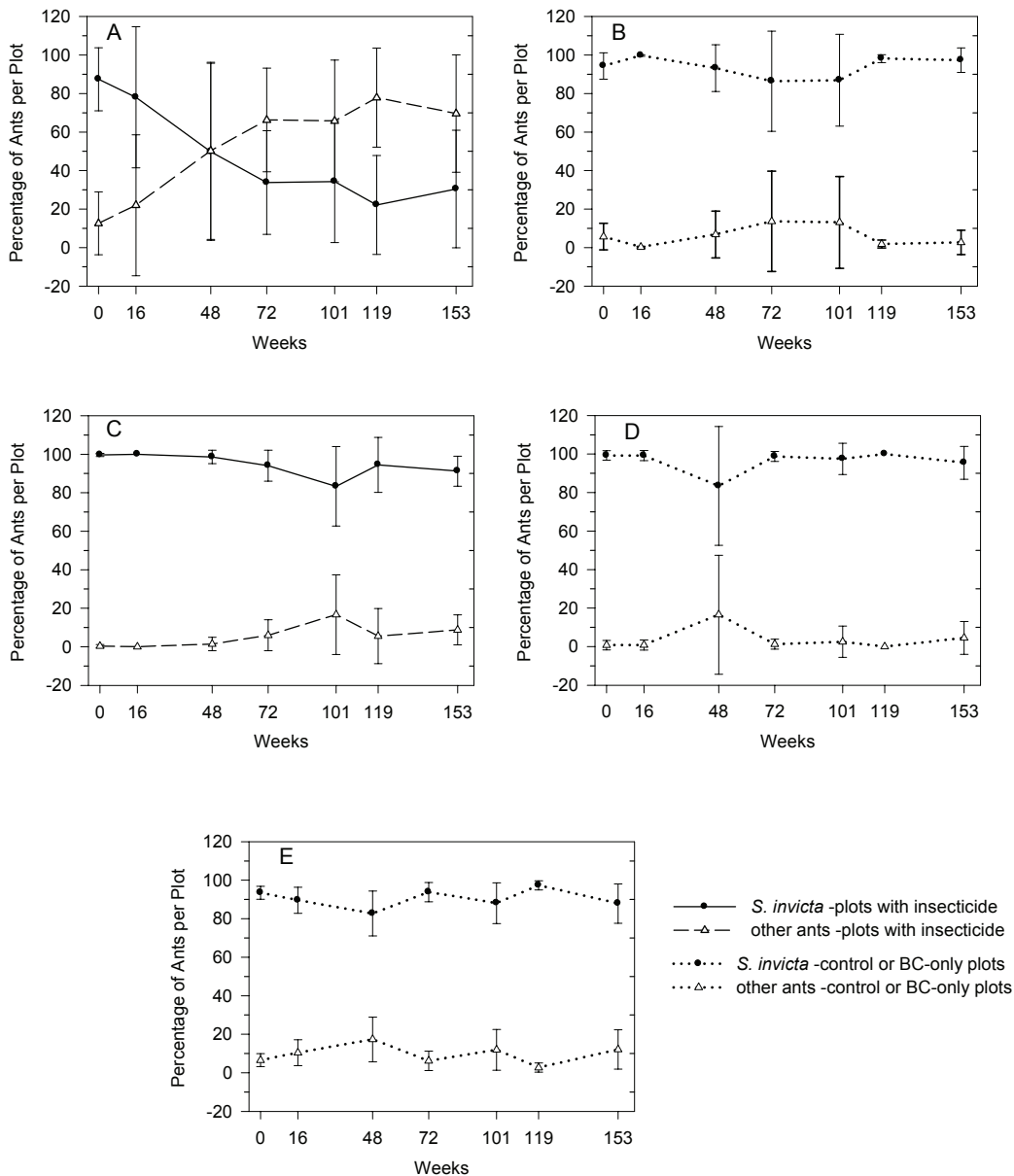
The number of ant genera among the non-*S. invicta* ants collected in pitfalls was highest in the untreated site with 12, followed by 11 in the insecticide+BC plots, 8 in the insecticide-only chemical-site plots, 6 in the BC-only plots, and 3 in the control chemical-site plots (Table 3). (Table 3 here) Totaled over all sampling dates, *Dorymyrmex* was the most abundant, accounting for 62 (982/1584), 68 (127/188), and 44% (438/1008) of non-*S. invicta* ants in the integrated, chemical, and untreated sites, respectively. Other genera collected with abundance greater than 100 summed over all sites include *Formica* (300 ants), *Crematogaster* (266), *Paratrechina* (213), *Forelius* (140), and *Pheidole* (115). After plots received the insecticide treatments, *Dorymyrmex* was the most prevalent non-*S. invicta* ant collected from the integrated site (84%) and the chemical site (83%). *Dorymyrmex bureni* (Trager) [= *Conomyrma insana* (Buckley)] preys upon fire ant reproductives landing after mating flights (Nickerson et al. 1975) and was found in areas with well established *S. invicta* infestations (Camilo and Philips 1990, Wojcik 1994). Abundance of various *Dorymyrmex* (= *Conomyrma*) species has been reported to increase after *S. invicta* populations were reduced with the application of fire ant baits (Markin et al. 1974, Zakharov and Thompson 1998).

The relatively similar percentage change in non-*S. invicta* ants between the insecticide+BC and BC-only plots in the integrated site suggested that the fipronil treatment was not completely suppressive to other ants, despite the very substantial decrease in *S. invicta* populations. Abundance of non-*S. invicta* in the chemical site was

generally low in both insecticide and control plots, before and after treatments. However, the average of 14 non-*S. invicta* ants on week 101 in the treated plots also indicated that other ants were present in the treated plots and not completely suppressed by the insecticide. The greater abundance and diversity of non-*S. invicta* ants in the insecticide+BC plots before treatment application among all other insecticide and control areas most likely contributed to the higher levels of non-*S. invicta* ants. A similar pattern of more non-*S. invicta* ants reestablishing after mirex bait applications occurred when pre-treatment diversity was greater (Summerlin et al. 1977).

**Site Comparisons.** The establishment and spread of fire-ant-specific pathogens and parasitoids at the integrated-site was extensive, yet fire ant populations in the surrounding BC-only plots were still present, with an average of 542 ( $\pm 155$  SD) nests per ha (219.5 nests/ac) over the last two sampling dates. This density of fire ants would be beyond acceptable fire ant population levels in areas where human or domesticated animal activity is high and reduction of fire ant stinging incidents is a priority. Thus, in this study, the reported levels of fire ant biological control agents by themselves would not have reduced fire ant populations sufficiently. However, direct, fast control of fire ant populations, similar to that of insecticides, is not a realistic objective for the biological control agents. Both *P. tricuspis* and *T. solenopsae* may have their most significant impacts on fire ants indirectly, for example, through foraging disruption (Porter et al. 1995) and debilitation of fire ant queens with consequent reduction in brood production (Williams et al. 1999). Establishment of new fire ant colonies is also hindered when they are infected with *T. solenopsae* (Cook et al. 2003, Oi and Williams 2003). Possible evidence of this phenomenon may be the disappearance of infected nests in the treated plots of the integrated site from weeks 72-119 (Fig. 2). Re-infestation in areas treated with fire ant bait containing hydramethylnon also has been observed to be slower in a *T. solenopsae*-infected area relative to an uninfected site (D. H. O. unpublished data).

An objective of this study was to determine if the establishment of fire ant biological control agents would result in reduced pesticide



**Fig. 3.** Average percentages and 95% confidence intervals per plot of *S. invicta* and non-*S. invicta* ants in pitfall traps on each sampling week after insecticide application /biocontrol releases of among a) integrated site – insecticide+BC plots; b) integrated site – BC-only plots; c) chemical site – insecticide-only plots; d) chemical site – control plots; e) untreated site plots.

use/risk by extending acceptable levels of control. Acceptable fire ant control depends on the use-pattern for a particular site (Drees et al. 1998, Drees et al. 2006). We designated a hypothetical re-treatment threshold of <90% control of the initial PIs. This threshold was based on the 49 nests/ha density in Oi et al. (1994), where fire ants did not dominate food lures relative to other ant species and the conservative nest density of 470 nests/ha for polygynous fire ant populations reported by Macom and Porter (1996)  $[(470-49)/470 = 0.90]$ . Thus, while the chemical site would have been retreated at week 72, the integrated site would not have needed another chemical application for at least the 3 years of this study. The spatial and temporal consistency of control was much higher in the integrated site, where the same single subplot had less than 90% reduction on 4 sampling dates during the study. In contrast, for the chemical site, 4 plots were less than the 90% control threshold on week 72; and for the entire study, fire ant populations crossed the control threshold on 28 occasions, encompassing all plots at least once (Fig. 2). However, if insecticides were actually reapplied, the frequency at which 90% control in individual plots was not obtained would most likely be less.

The granular contact insecticide containing fipronil provided

control that extended much longer than traditional treatments of broadcast applications of fire ant baits. Depending on factors such as the active ingredient, season applied, and proximity of surrounding fire ant populations, baited areas typically would begin to be re-infested in 3 - 12 months (Collins et al. 1992, Barr et al. 2005). In contrast, granular fipronil applied to surfaces will continue to kill colonies for a year (Barr and Best 2002, Drees et al. 2006), and control achieved at the chemical-site was slightly less than that reported by Collins et al. (2000), where at 99 wk, 79% control was obtained. The  $\geq 95\%$  control for nearly 3 years at the integrated site exhibited a potential of fire ant biological control agents to extend control and subsequently reduce pesticide use. Assuming that the chemical site would have been retreated at week 72 and subsequently achieved the same duration of control, the amount of insecticide applied would be double that of the integrated site. Of course, in determining pesticide use/risk reduction, other aspects such as toxicity and residual exposure, in addition to the amount of active ingredient applied, should be considered (Drees 2003).

The release, establishment, and spread of fire ant biological control agents in the U.S. have occurred and are continuing. Because of

**Table 2. Number of *S. invicta* and other ants per plot-sample date collected in pitfall traps among treatments within sites where fire ant biological controls were released and insecticide treatments applied (Integrated site), treated with insecticide only (Chemical site), and where biological or chemical controls were not used (Untreated site), Richland County, South Carolina.**

Site: Treatment	Ant	Avg. No. Ants Plot ( $\pm$ Std. error)						
		Weeks after treatment						
		0	16	48	72	101	119 <sup>a</sup>	153
Integrated: Insecticide+BC	<i>S. invicta</i> (n=8)	1946.3 (799.5)	67.6 (23.8)	16.9 (13.3)	21.8 (10.4)	38.4 (15.9)	3.3 (1.6)	5.4 (2.8)
	Other ants (n=8)	42.8 (24.2)	3.0 (2.4)	4.1 (2.5)	19.5 (3.5)	49.8 (13.9)	4.9 (0.5)	10.3 (4.3)
Integrated: BC-only	<i>S. invicta</i> (n=8)	1541.9 (635.6)	1051.5 (250.2)	295.4 (62.7)	388.8 (106.7)	793.8 (275.8)	148.9 (37.9)	310.8 (74.8)
	Other ants (n=8)	18.6 (10.1)	1.1 (0.6)	12.5 (10.3)	8.4 (5.0)	21.3 (13.1)	1.5 (0.9)	0.4 (0.4)
Chemical: Insecticide	<i>S. invicta</i> (n=8)	459.75 (107.44)	23.63 (5.84)	3 (1.04)	8.63 (1.28)	71.75 (30.49)	1.13 (0.30)	41.88 (14.50)
	Other ants (n=8)	1.63 (1.49)	0.00 (0.00)	0.13 (0.13)	0.75 (0.49)	14.38 (9.37)	0.13 (0.13)	3.38 (1.44)
Chemical: Control	<i>S. invicta</i> (n=4)	279.25 (98.23)	118.5 (76.00)	22.25 (17.62)	80.75 (60.89)	26 (6.36)	8.5 (3.30)	53.5 (10.67)
	Other ants (n=4)	0.50 (0.50)	0.25 (0.25)	1.00 (0.41)	1.50 (1.19)	1.25 (1.25)	0.00 (0.00)	1.75 (0.75)
Untreated	<i>S. invicta</i> (n=12)	154.42 (31.09)	105.92 (21.83)	91.58 (21.62)	117.08 (18.72)	271 (71.94)	94.42 (22.20)	89.5 (20.49)
	Other ants(n=12)	7.17 (1.31)	21.92 (14.49)	19.25 (7.47)	6.25 (2.22)	17.58 (5.81)	1.17 (0.37)	10.75 (4.54)

<sup>a</sup> Number of ants trapped reduced by rain

their indirect effect on fire ant populations, it may take many years and more agents to detect a measurable effect (Morrison and Porter 2005b) and a perceivable, regional impact. With the widespread dispersal of the fire ant decapitating phorid flies and the increasing prevalence of *T. solenopsae* in polygynous fire ant populations (Oi et al. 2004, D. H. Oi and R. M. P. unpublished data, Mitchell et al. 2006), determination of impact is becoming increasingly difficult because of the lack of replicated control sites. Soon, comparisons may go the way of some of the early biological control introductions where impact was evaluated by pre- and post-introduction data and observations (Debach et al. 1976). Optimistically, future research may document self-sustaining and complete control such as seen with the classical biological control of the sugarcane leafhopper in Hawaii, which occurred after 20 years of introducing several natural enemies (Clausen 1978), or much faster control exemplified by the discovery and introduction of the *Oryctes* virus against the palm rhinoceros beetles in several south Pacific islands (Bedford 1980). In the case of fire ants, the low or even absent tolerance for stings in sensitive areas, such as childcare playgrounds and nursing homes, represents a need to take an integrated pest management approach to reap the

potential benefits of biological control. The intensity and location of the chemical control efforts should reflect a realistic tolerance for sting incidents. Monitoring for re-infestations and limiting treatment to infested, sensitive areas can reduce insecticide use for fire ants and perhaps facilitate the survival of biological control agents.

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**Table 3. Genera, total number, and percentage of non-*S. invicta* ants in pitfall traps from plots within the Integrated, Chemical, and Untreated sites before insecticide was applied and biological controls released (week 0), and in the fall and spring for 3 years.**

Sample Period	Genus, Total Number and (% of non- <i>S. invicta</i> Ants)				
	Integrated-Site		Chemical-Site		Untreated-Site
	BC <sup>a</sup> +Insecticide (n=8 <sup>b</sup> )	BC-only (n=8)	Insecticide (n=8)	Control (n=4)	Control (n=12)
Pre-Treatment	<i>Crematogaster</i> 226 (66.1)	<i>Dorymyrmex</i> 83 (55.7)	<i>Brachymyrmex</i> 7 (53.8)	<i>Paratrechina</i> 2 (100)	<i>Paratrechina</i> 32 (37.6)
June 2000	<i>Lasius</i> 62 (18.1)	<i>Crematogaster</i> 28 (18.8)	<i>Lasius</i> 3 (23.1)		<i>Forelius</i> 27 (31.8)
(week 0)	<i>Dorymyrmex</i> 26 (7.6)	<i>Pheidole</i> 15 (10.1)	<i>Dorymyrmex</i> 2 (15.4)		<i>Dorymyrmex</i> 10 (11.8)
	<i>Brachymyrmex</i> 11 (3.2)	<i>Paratrechina</i> 13 (8.7)	<i>Paratrechina</i> 1 (7.7)		<i>Formica</i> 7 (8.2)
	<i>Formica</i> 5 (1.5)	<i>Brachymyrmex</i> 5 (3.4)			<i>Solenopsis (Diplo.<sup>c</sup>)</i> 3 (3.5)
	<i>Paratrechina</i> 4 (1.2)	<i>Forelius</i> 5 (3.4)			<i>Camponotus</i> 2 (2.4)
	<i>Pheidole</i> 3 (0.9)				<i>Brachymyrmex</i> 1 (1.2)
	<i>Solenopsis (Diplo.<sup>c</sup>)</i> 3 (0.9)				<i>Hypoconera</i> 1 (1.2)
	<i>Forelius</i> 2 (0.6)				<i>Pheidole</i> 1 (1.2)
					<i>Pyramica</i> 1 (1.2)
Post-Treatment Spring	<i>Dorymyrmex</i> 466 (90.8)	<i>Dorymyrmex</i> 183 (67.0)	<i>Dorymyrmex</i> 119 (83.2)	<i>Paratrechina</i> 13 (81.3)	<i>Formica</i> 251 (44.0)
	<i>Pheidole</i> 30 (5.8)	<i>Forelius</i> 61 (22.3)	<i>Brachymyrmex</i> 11 (7.7)	<i>Formica</i> 2 (12.5)	<i>Dorymyrmex</i> 197 (34.5)
May 2001-2003	<i>Crematogaster</i> 7 (1.4)	<i>Pheidole</i> 14 (5.1)	<i>Paratrechina</i> 6 (4.2)	<i>Hypoconera</i> 1 (6.3)	<i>Paratrechina</i> 69 (12.1)
(weeks 48, 101, 153)	<i>Brachymyrmex</i> 3 (0.6)	<i>Paratrechina</i> 8 (2.9)	<i>Forelius</i> 3 (2.1)		<i>Forelius</i> 39 (6.8)
	<i>Paratrechina</i> 3 (0.6)	<i>Crematogaster</i> 5 (1.8)	<i>Hypoconera</i> 2 (1.4)		<i>Solenopsis (Diplo.<sup>c</sup>)</i> 7 (1.2)
	<i>Aphaenogaster</i> 2 (0.4)	<i>Brachymyrmex</i> 2 (0.7)	<i>Formica</i> 1 (0.7)		<i>Hypoconera</i> 4 (0.7)
	<i>Hypoconera</i> 1 (0.2)		<i>Pheidole</i> 1 (0.7)		<i>Pheidole</i> 2 (0.4)
	<i>Trachymyrmex</i> 1 (0.2)				<i>Aphaenogaster</i> 1 (0.2)
					<i>Camponotus</i> 1 (0.2)
Post-Treatment Fall	<i>Dorymyrmex</i> 150 (68.5)	<i>Dorymyrmex</i> 74 (84.1)	<i>Dorymyrmex</i> 6 (85.7)	<i>Hypoconera</i> 6 (85.7)	<i>Dorymyrmex</i> 231 (65.6)
	<i>Pheidole</i> 44 (20.1)	<i>Paratrechina</i> 7 (8.0)	<i>Pheidole</i> 1 (14.3)	<i>Paratrechina</i> 1 (14.3)	<i>Paratrechina</i> 40 (11.4)
Sept./Oct. 2000-2002	<i>Paratrechina</i> 14 (6.4)	<i>Pheidole</i> 4 (4.5)			<i>Formica</i> 34 (9.7)
(weeks 16, 72, 119)	<i>Brachymyrmex</i> 9 (4.1)	<i>Brachymyrmex</i> 3 (3.4)			<i>Solenopsis (Diplo.<sup>c</sup>)</i> 18 (5.1)
	<i>Hypoconera</i> 2 (0.9)				<i>Neivamyrmex</i> 16 (4.5)
					<i>Hypoconera</i> 7 (2.0)
					<i>Forelius</i> 3 (0.9)
					<i>Aphaenogaster</i> 1 (0.3)
					<i>Camponotus</i> 1 (0.3)
					<i>Pyramica</i> 1 (0.3)

<sup>a</sup> BC, Biocontrol

<sup>b</sup> number of plots

<sup>c</sup> *Diplo.*, *Diplorhoptrum*

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**NOTE:** Please add short bio for each author.