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IMPORTED FIRE ANT: HISTORY, IMPACT AND MANAGEMENT

David F. Williams

USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology
1600/1700 SW 23rd Drive
Gainesville, FL 32608

HISTORY
The red imported fire ant, Solenopsis invicta Buren, was probably introduced in the port of Mobile, AL around 1930's. The first collections were obtained in Mobile and the 1st identification was made as S. saevissima var. richteri in 1930. In 1930, the infestation was limited to the Mobile area. The red imported fire ant continued to spread by means of mating flights, the movement of colonies and newly mated queens on flood waters, movement of nursery stock, bee hives, construction equipment, railroad cars and open truck beds. In 1949, a survey found 14 Mississippi, 12 Alabama and 2 Florida counties infested and then in 1953, a four year survey by the USDA showed that this ant had spread to 102 counties in 10 states. The increased range of the red imported fire ant is now over 321 million acres in southern U.S. and Puerto Rico. The principal areas of expansion include Oklahoma, Arkansas, Tennessee, Texas, North Carolina, and Virginia with recent infestations occurring in California, New Mexico, and Arizona.

IMPACT
The impact of S. invicta is extensive and occurs on human health, crops, livestock, wildlife, biodiversity, utilities, recreation, tourism, transportation (roadways), and environmental quality. The most noticeable problem caused by fire ants is stinging of humans which in some cases cause serious injuries and can even result in death. S. invicta is also responsible for damage to farm products such as soybeans, okra, potatoes, corn, and young citrus trees. It also impacts on the nursery industry because of the federal quarantine requirement that all nursery stock and grass sod moving out of the fire ant infested areas be treated with approved insecticides. In addition, S. invicta is a threat to domestic animals causing economic losses to farmers and can cause an overall reduction in biodiversity in many areas. The overall impact from S. invicta infestations across the southern U.S. is over $1 billion dollars annually.

MANAGEMENT
There are several reasons why S. invicta are difficult to control. For example, they have large populations, produce large numbers of reproductives, lack natural enemies, have little competition, are very aggressive and sting, and thrive in a wide range of habitats. During the World War II years (1940-1947) all control programs for S. invicta were halted and this ant continued to spread. In 1948, Mississippi, Alabama, & Louisiana appropriated funds for control programs using 5% chlordane dust. In 1957, the U.S. Congress appropriated $2.4 million to USDA to initiate a federal/state cooperative control and eradication program. In November, 1957, heptachlor and dieldrin were applied by air and ground equipment and within one year, environmental concerns began because of high mortality seen in birds. During the 1960's, mirex bait was developed by the USDA-ARS and in 1962, mirex bait replaced heptachlor as the treatment for S. invicta. Mirex was applied from 1962-1978 using converted World War II
aircraft over more than 140 million acres. Total treatment cost was 30 cents per acre which included the bait and application. In the late 1960's, mirex residues were detected in nontarget organisms and all registrations of mirex were cancelled in 1978. The other reasons for cancellation were persistence in the environment, accumulation in nontarget organisms, toxicity to estuarine organisms and its potential as a carcinogen. Following the ban of mirex, political pressure on Congress for finding a replacement was intense, thus, Congress appropriated funds for the USDA-ARS to begin an intensified search for new chemicals for use in fire ant baits. This allowed an increase in the number of chemicals evaluated for S. invicta baits by USDA-ARS. During 1958-1976 (18 years), 2,678 chemicals were evaluated, however, from 1977-1988 (11 years), 4,432 products were tested. The historical development of chemicals for fire ant control is as follows:

-1937 calcium cyanide dust
-1947 chlordane dust
-1957 heptachlor and dieldrin granulants
-1962 - 1978 mirex
-1980's hydramethylnon, fenoxycarb, and abamectin
-1990's pyriproxyfen, methoprene, and spinosad
-2000's fipronil

Baits for S. invicta consists of the following components, (1) attractants (oils, sugars, proteins, and insects), (2) toxicants (metabolic inhibitors—i.e. hydramethylnon; IGR’s—i.e. fenoxycarb; reproductive inhibitors—i.e. abamectin; chitin synthesis inhibitors—i.e. teflubenzuron), and (3) carriers—(corn grits, sawdust, clay or mineral granules, and insects). The criteria needed for optimum chemical use in S. invicta baits are:

-Delayed Toxicity
-Effective over wide dosage range
-Formulates with food and carriers easily
-Not repellent to ants
-Environmentally safe

The present control of S. invicta consists mainly of using chemicals. The chemical control options are the use of baits, contact insecticides (sprays, drenches, dusts, granules, etc.) and combinations of these two. Present-day fire ant baits generally give 80-95% control within 2 wks – 3 months and last 4 – 12 months after application. Most baits are applied at 1 – 1.5 lbs per acre and the costs of the bait products range from about $7.00 - $12.00 per lb.

In the mid-1970’s, there was an increase in surveys for natural control agents for S. invicta in South America. Self-sustaining biological control agents could become a major factor in providing long term suppression of fire ant populations, something that chemicals do not offer. Also, fire ant densities are more than 5 times higher in the U.S. than in their native South America and the only differences appear to be the natural enemies in South America. With more than 30 natural enemies of fire ants having been discovered in South America, several investigators began concentrating their research efforts towards searching for potential biological control agents for use against S. invicta.

Several biological control agents are presently being studied such as the parasitic ant, Solenopsis daguerrei, decapitating phorid flies, Pseudacteon tricuspid, P. curvatus, and P. littoralis, and
protozoans, *Thelohania solenopsae, Vairimorpha invictae*, and *Mattesia* sp. In addition, studies have also been conducted with the fungus, *Beauveria bassiana*.

**SUMMARY**
The red imported fire ant, *S. invicta*, infest over 321 million acres in the U.S., and are an increasing problem having a considerable impact. Chemicals are still the most effective method of control but self-sustaining biological control agents could provide long term suppression of fire ant populations. Future control should involve multiple strategies of chemical, biological, behavioral, molecular, physical, and cultural methods. Research and education are important keys and will play vital roles in the future management of this pest.
HOST LOCATION BEHAVIOR IN A PARASITOID OF IMPORTED FIRE ANTS

L. W. Morrison¹ & J. R. King¹²

¹Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, P.O. Box 14565, Gainesville, FL 32604 USA

²Entomology and Nematology Department, University of Florida, Gainesville, FL 32611

ABSTRACT. Imported fire ants (Solenopsis invicta and S. richteri) are serious pests in the southeastern U.S. The release of natural enemies as biological control agents may reduce the abundance of these pests. Species of parasitoid flies in the genus Pseudacteon (Diptera: Phoridae) are currently being released and evaluated at selected field sites. We studied the mechanisms by which one of these species, P. tricuspis, locates its host, S. invicta, in a series of field observations and experiments. The parasitoids were frequently attracted to host workers at disturbed colonies, but were almost never attracted to host workers foraging at baits. When conspecific non-nestmate workers were introduced to baits, resulting in aggressive interactions, parasitoids appeared at the majority of baits. Moreover, larger numbers of parasitoids appeared at baits to which greater numbers of non-nestmate workers had been added. Addition of non-nestmate workers to disturbed colonies resulted in increased numbers of parasitoids attracted. Pseudacteon tricuspis is apparently attracted to the alarm pheromones released by the ants involved in aggressive interactions or colony disturbances, and not to recruitment pheromones released when foraging. Comparisons with other studies suggest that not all Pseudacteon ant parasitoids use the same host location cues, or at least not to the same degree. Pseudacteon tricuspis is likely to be relatively more effective as a biocontrol agent in areas with frequent mound disturbances or more competing ants. This study indicates the need to release other Pseudacteon species with complementary host location behaviors.
SYMPATRY OF FIRE ANT POLYGYNE AND MONOGYNE SOCIAL FORMS

R. K. Vander Meer\(^1\) and G. N. Fritz\(^2\)

\(^1\)USDA/ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23\(^{rd}\) Dr., Gainesville, FL 32608; \(^2\)Eastern Illinois University, Department of Biological Sciences, Charleston, Illinois 61920

Abstract. The polygyne form of the red imported fire ant, *Solenopsis invicta*, had been thought to occur primarily in discrete and homogeneous populations within areas composed of monogyne colonies. Polygyne queens are inseminated primarily by monogyne-derived males. Therefore, opportunities for female alate insemination might be minimal at the centers of large polygyne populations. We tested the homogeneity of a large polygyne population in North central Florida by examining colonies at six sites located along an East-West transect through this polygyne population. The social form of each colony sampled was determined by an aggression test of workers to the introduction of non-nestmates and by the dissection of males for sterility. Both social forms of *S. invicta* were present at all collection sites. About 30\% of all colonies sampled (N = 333) were determined to be monogyne. The polygyne region in North central Florida is more accurately described as an area where relatively high frequencies of polygyne colonies(7,5),(995,987)

Introduction. Patches of polygyne fire ant colonies have been reported in many areas of the Southeast, including Louisiana, Florida, Arkansas, Alabama, Oklahoma, and Texas (Fletcher et al. 1980, Mirenda & Vinson 1982, Lofgren & Williams 1984, Ross & Fletcher 1985a, Glancey et al. 1987, Glancey et al. 1989). Studies on the densities and distribution of polygyne colonies in the Southeast have implied that certain areas are exclusively inhabited by polygyne colonies (e.g. Porter et al. 1991). The manner in which these regions became polygyne is not clear.

A region of North Florida has one of the largest polygyne patches of fire ants in the United States (Porter 1992), encompassing approximately 2900 km\(^2\). Porter (1992) sampled 113 different locations in this particular polygyne patch in 1991 and re-sampled 103 of these sites in 1992 (Porter 1993). The distribution map of both social forms indicates that single queen colonies are absent from most areas where polygyne colonies have been collected (Porter 1992, 1993). A similar exclusion of monogyne colonies from polygyne areas has been noted in Texas (Porter et al. 1991). However, Ross (1992) reported that over 80\% of polygyne queens mate with single queen males, thus requiring the participation of both social forms in the same mating flights. The purpose of this study was to determine whether the large polygyne region of Florida was exclusively polygyne, as previously indicated, or had low frequencies of monogyne colonies as well. We examined the distribution of both social forms along an East-West transect through the center of the polygyne patch in North central Florida.

Materials and Methods. Six collection sites were established along an East-West transect in north central Florida (Figure 1). The western-most end of transect started in Levy County and continued eastward to the easternmost edge of Marion County (Figure 1, B-G). Up to 51 separate colonies of *S. invicta* were sampled at each collection site. The distance between mounds was at least 10m. The top portion of a colony was removed and several hundred workers plus nest soil were collected from each colony. When males were found they were collected and later dissected for the presence of spermatozoa. All colony samples were transported to a laboratory facility for behavioral assays.
Figure 1. Location of seven collection sites of *S. invicta* in north central Florida; East-West transect bisects the center of the polygyne population (sites B-G). The three collection locations and identity (polygyne or monogyne) of colonies from site A are shown at the right.

Two methods were used to establish the social form of colonies. One method was an assay quantifying the aggressive behavior of workers towards non-nestmate intruders (Morel et al. 1990). The second method was dissection of males for gonadal development, since only polygyne colonies are expected to produce sterile males (Ross & Fletcher 1985b, 1986; and see Hung et al. 1974, for the method).

**Results.** The distribution of initial aggression scores was bimodal (Figure 2), corresponding to the two social forms (monogyne aggressive and polygyne non-aggressive, Morel et al. 1990). Twenty-two colonies initially scored between the monogyne criteria (≥7) and the polygyne criteria (≤4). When these colonies were re-tested, four scored in the monogyne range and the remaining 18 scored in the polygyne range. The combined results were used to designate colonies as either monogyne or polygyne.

The determination of the sterility of males collected from 20 colonies and six sites was consistent with the determination of social form by the behavioral bioassay. From one to eight males were collected from 20 colonies in six of the collection sites. Nine of these colonies were determined to be of single queens based on the aggression test and all but one of the males (n = 35) from these colonies was fertile. Seven of the 11 colonies scored as polygyne by the aggression test had one or more sterile males (12 of 25 sterile).
Discussion. Polygyne colonies produce significantly fewer sexuals per unit time than those with single queens (Vargo & Fletcher 1987). In addition, polygyne colonies primarily produce sterile males (Ross & Fletcher 1985b). Polygyne-derived female alates appear to depend primarily on monogynie colony-derived males for successful mating, since males from monogynie colonies account for approximately 80% of polygynie queen inseminations (Ross 1997). Therefore, polygynie colonies must participate in monogynie mating flights and polygynie colonies that are far removed from sources of monogynie males may have difficulty maintaining numbers of fertile queens by queen adoption or difficulty expanding their territory through budding. Such large tracts of polygynie colonies have been reported for both Texas (Porter et al. 1991) and Florida (Porter 1992, 1993).

Our data demonstrate that the North Florida transect bisects an area that is primarily polygynie, as expected from previous surveys (Porter 1992, 1993). However, the number of monogynie colonies found at each site was unexpected. Monogynie colonies were found at every site along the transect and comprised approximately 25% or more of the colonies encountered at 5 of the 6 sites. Polygynie, therefore, does not inevitably lead to the complete displacement of monogynie colonies in a particular region. Most likely, though, monogynie colonies persist in polygynie areas by foundress queens originating from local monogynie colonies or from those in adjacent areas. Newly mated queens from polygynie colonies have relatively small fat reserves and are not expected to be successful at independent colony foundation (Porter et al. 1988, Keller & Ross 1993, 1995). Polygynie colonies are thought to spread primarily by budding (Vargo & Porter 1989). Although the literature on S. invicta implies that the polygynie form has a negative effect on the presence and density of the monogynie form, the proximate reasons for such an effect are not known and the relationship remains speculative. If there is a
competitive interaction between both social forms in Florida, the vagility of single queen forms probably helps maintain their presence in available habitat.

We have demonstrated that monogyne colonies can be found in close association with polygyne *S. invicta* populations, which probably maintains the reproductive and genetic dynamics between both social forms as reported by Ross (1997, Ross & Fletcher 1985a, Ross et al. 1996). While our data is for a Florida population our conclusions are logically extended across the fire ant range in the United States.

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**References Cited**


PREDATION OF RED IMPORTED FIRE ANT ALATES IN CENTRAL GEORGIA

Reid Ipser, Hal Peeler, and Wayne Gardner

Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, 1109 Experiment Street, Griffin, GA 30223

Introduction. The effects of non-indigenous organisms on an ecosystem are at least partially determined by certain intrinsic characteristics of the invader (Diamond and Case 1986), including reproduction and foraging activities. The extent of the impact also can depend on whether the organism invade an empty niche, thereby having little impact, or whether it displaces species from occupied niches (Walker and Valentine 1984, Herbold and Moyle 1986). In addition, the abiotic environment frequently sets limits on what habitats a species can invade successfully. Thus, there is often a similarity between the abiotic environment of the native home range of an exotic pest and its expanded range (Simberloff 1986, Brown 1989, Crawley 1986).

Founding queens of *S. invicta* are subject to high levels of predation during nuptial flights and prior to entering the soil. The majority of founding queens are reportedly killed by *Solenopsis* spp. during this period (Whitcomb et al. 1973, Carroll and Janzen 1973). Moreover, 99% of *S. invicta* queens are killed by predators, including subterranean predators of the genus *Solenopsis* (Diplorhoptrum) (Whitcomb et al. 1973, Buren 1983). Buren (1983) further indicated that *Diplorhoptrum* species could be potentially used in suppressing *S. invicta* populations in the U.S. because of their subterranean habits.

The objective of this study was to examine and compare predation rates of red imported fire ant alates in canopied and un-canopied areas to (1) identify potential natural regulators of *S. invicta*, and (2) estimate the potential impact of predation in these two habitats

Procedures. The study was conducted at two state parks located in south-central Georgia (High Falls, Indian Springs). Each of these parks contains more than 200 ha of wooded areas characterized as second-growth forests comprised of oak, loblolly pine, slash pine, and other softwood and hardwood species. They also include over 100 ha of open areas. Sampling sites in the second-growth-forests were located 60 m from right-of-ways or open mads and encompassed an area of 1000 m² each. Adjacent open fields, characterized as frequently disturbed fields (mowed once a month) containing primarily grasses, were used as comparison sample sites. The area of each open field sampled also encompassed 1000 m². Each site was sampled once a month.

Mortality of fire ant reproductives was assessed in each habitat. *Solenopsis invicta* reproductives (alates) in plastic vials were buried in the ground to determine predation rates. Alates were collected from Tab colonies in Griffin, GA. One individual alate was placed in each of twenty 20-ml plastic vials. The lid of each vial was modified by cutting a hole (=2 cm diam) through the center (Nichols and Sites 1991). Wire screen (1.66 mm² mesh) was placed over each vial opening and was held in place by the modified lid. This screen was designed to allow workers of most ant species and other small arthropods to enter the vial while preventing the escape of the alates (Nichols and Sites 1991). Control vials were similar in construction, but a 0.8 mm² cover
was placed over each vial opening to exclude all arthropod predators. The bottom of each vial contained dental plaster that was moistened to prevent desiccation of the alate.

Ten experimental and ten control vials were placed 2 m apart and 10 cm deep in the soil in a linear transect. Each was covered with a rock or piece of wood to simulate colony founding. Vials were checked every day for 7 days. Alates were reported as alive or preyed upon. Species of ants or other predators present in vials were collected and placed in 70% ETOH. Differences in S. invicta alate mortality were used as indicators of the degree of founding success in the different habitats.

A $X^2$ test was used to determine if there was a significant difference in alate predation.

**Results and Discussion.** A chi-squared analysis indicated that predation rates were significantly different in the canopied and un-canopied areas ($X^2 = 145.729; df = 1$). Higher rates of predation occurred in canopied areas as compared to un-canopied areas (Fig 1). Alates in the control vials experienced lower rates of death as compared to the ants in the “exposed to potential predation vials” (Fig 2). Furthermore, predation was slightly higher in High Falls State Park as compared to Indian Springs (Fig 3), and there was a decrease in predation from the months of September through December (Fig 4). Ants collected in vials are listed in Table 1. We feel that in the months measured so far, *Prenolepis impairs* and *S. invicta* were the primary predators in the canopied and un-canopied habitats, respectively, for each area studied at each state park.

We feel that as the landscape of Georgia state parks continues to be altered for the purpose of fashioning additional recreation areas or for rehabilitating unused areas, the future work of this study will promote understanding of native ant predatory behavior and provide insight into applications focused on inhibiting red-imported-fire-ant founding of alates.

**References Cited**


Diamond, J. and T. J. Case. 1986. Overview: Introductions, extinctions, exterminations,


Table 1. Predator Ant Species Found in Vials

<table>
<thead>
<tr>
<th>Species</th>
<th>Canopied/Un-canopied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphaenogaster picea</td>
<td>(canopied)</td>
</tr>
<tr>
<td>Crematogaster lineolata</td>
<td>(canopied)</td>
</tr>
<tr>
<td>Monomorium minimum</td>
<td>(canopied and un-canopied)</td>
</tr>
<tr>
<td>Prenolepis impairs</td>
<td>(canopied)</td>
</tr>
<tr>
<td>Pyramica sp.</td>
<td>(canopied)</td>
</tr>
<tr>
<td>Solenopsis invicta</td>
<td>(un-canopied)</td>
</tr>
<tr>
<td>Solenopsis molesta</td>
<td>(canopied)</td>
</tr>
</tbody>
</table>
Figure 1: Percent Predation in Response to Habitat

Figure 2: Percent Predation in Response to Screen Mesh Size
Figure 3: Percent Predation in Response to Site (State Park)

Figure 4: Percent Predation in Response to Date
LONG TERM EFFECT OF THE FIRE ANT PATHOGENS VAIRIMORPHA INVICTAE AND THELOHANIA SOLENOPSAE IN ARGENTINA

J. A. Briano¹, D. F. Williams², and D. H. Qi²

¹ USDA-ARS-South American Biological Control Laboratory, Hurlingham, Argentina
² USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL

Abstract

A long term study of fire ant populations (mainly Solenopsis invicta Buren) naturally infected with Vairimorpha invictae Jouvenaz and Ellis (Microsporida: Burendidae) and Thelohania solenopsae Knell, Allen, and Hazard (Microsporida: Thelohaniidae) is in progress. Six rectangular field plots (10 x 100 m) were set up in north central Santa Fe Province, Argentina, in May 2000. Initially, five of these plots were infected with microsporidia, the density of colonies ranged from 4 to 19 per plot and the infection rates ranged from 15 to 50% of the active colonies. The other plot was infection-free (control plot) with an initial density of 8 uninfected colonies. The plots have been monitored every 2-5 months for the density of colonies (mound counts and population indexes) and rates of microsporidian infections. In February 2001, a 85-100% reduction in fire ant colony density was observed in all infected plots. Some reinfestation occurred in two plots. In contrast, in the control plot, a gradual increase in colony density was observed until both microsporidia naturally infected the plot in December 2001. After that, a 100% reduction in colony density was observed. In February 2002, only 5% of the initial number of colonies was found in one plot. Preliminary conclusions indicate that these microsporidia have lowered the field density of S. invicta in Argentina. The magnitude of their impact will be determined at the end of the study (2003/2004). This detrimental effect on S. invicta populations is consistent with previous work conducted in Buenos Aires Province with S. richteri Forel. Because of dual infections, it was impossible to separate the individual effects of both microsporidia.
LIGHT INTENSITY AFFECTS DISTRIBUTION OF ATTACKING PSEUDACTEON CURVATUS (DIPTERA: PHORIDAE) IN A LABORATORY REARING SYSTEM

James T. Vogt

USDA-ARS-BCMRRU
P. O. Box 225
Stoneville, Mississippi 38776

Abstract

Distribution of imported fire ant attacking phorid flies (Pseudacteon curvatus Borgmeier) in a laboratory rearing system was tested for dependence on light intensity under 2 different light regimes. Light intensity (range, approx. 220-340 Lux) influenced fly distribution in light regime 1; in the second light regime, light intensity (range, approx. 300-390 Lux) had no effect. Results highlight the importance of testing distribution of attacking flies in phorid rearing systems and adjusting light configuration as necessary to optimize use of rearing space.

Introduction

Positive phototaxy leads to predictable behaviors in many insect species. These behaviors can be exploited for easy handling of live specimens, or for trapping and monitoring purposes. In laboratory rearing systems, however, it is often difficult to achieve even light distribution. If lighting gradients exist, positively phototaxic species may not distribute themselves evenly, and rearing space may not be optimally utilized.

Laboratory rearing of Pseudacteon flies that attack imported fire ants is accomplished by allowing adult flies to seek out and attack live ants in large attack boxes (Fig. 1). A series of ways within each attack box contain live host ants (Solenopsis richteri Forel, and Solenopsis invicta Buren x richteri), which are exposed to phorid attack for 3-4 d, then removed and held for parasitoid development. In the attack box, host ants are induced to trail back and forth within each tray by providing brood for them to carry, and alternately raising and lowering paired, inverted cups under which the ants seek shelter. By inducing ants to trail, "freezing" behavior (e.g., Porter et al. 1995, Cônsoli et al. 2001) is largely avoided and host ants are constantly exposed for attack.

P. curvatus flies are positively phototactic; thus, an experiment was conducted to examine their distribution in a rearing system at the USDA-ARS Biological Control and Mass Rearing Research Unit, Mississippi State, MS. The purpose of this work was to help optimize use of rearing space, and ultimately increase the number of flies reared in the system.

Materials and Methods

An attack box for rearing P. curvatus was constructed at the USDA-ARS, BCMRRU. Light was initially provided by three 2-tube, 75 W, 244 cm fluorescent fixtures (''daylight'' tubes) mounted above the box. Light intensity was measured in each tray using an Extech 407026 light meter
Estimates of fly activity were obtained by periodically counting the number of actively attacking flies / 15 sec observation / tray. Time of day was noted for each count. Fly activity data were placed in 4 time categories (1, 0900-1030 h; 2, 1031-1200 h; 3, 1201-1330 h; 4, 1331 + h). Data for each time category within each light configuration were subjected to Proc Mixed (Littell et al. 1996) to test for the fixed effects of light intensity and distance from point of emergence. Since adult flies entered the box from one end, distance (no. of trays) from that end was included as a variable to see if attacking flies stayed near the point of emergence or moving throughout the box. A second light configuration was tested, using additional fixtures (single-tube, 30 W, 91 cm fluorescent), one on each end of the attack box perpendicular to the main lights.

Fig.1. An attack box for rearing *Pseudacteon* spp., side view (A) and top view (B). Lights (not shown) were placed on brackets (1); additional fluorescent fixtures were placed across each end of the box. Phorid pupae were placed in an emergence box (2) and emerged into the attack box through a connecting hole (3). A pneumatic motor (4) alternately raised and lowered a pair of cups (5) in each tray.
Results and Discussion

Light intensity was lowest in end trays and highest in center trays for both light configurations (Fig. 2); however, the difference was ≈ 34% less with the additional lights. Distance from the point of adult fly emergence had no effect on fly attack ($P > 0.05$). Fly attack was influenced by light intensity in time categories 2 and 4, light regime 1, but not in light regime 2 (data summarized in Table 1).

![Fig. 2. Light intensity in an attack box for rearing the phorid fly *Pseudacteon curvatus*.](image)

Table 1. $F$ statistics for effect of light intensity on phorid fly distribution.

<table>
<thead>
<tr>
<th>Time category</th>
<th>Light regime</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>0.01</td>
<td>0.908</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.49</td>
<td>0.485</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5.85</td>
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<tr>
<td>2</td>
<td>2</td>
<td>1.27</td>
<td>0.733</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2.13</td>
<td>0.147</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.58</td>
<td>0.447</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>8.20</td>
<td>0.005</td>
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<tr>
<td>4</td>
<td>2</td>
<td>0.64</td>
<td>0.425</td>
</tr>
</tbody>
</table>
Surface plots of fly attack rates in space and time reveal trends within the rearing system (Fig. 3). The general decline in attack from the rear of the plots (early in the day) to the front (late in the day) reflects fly mortality and behavioral periodicity. While fly attack rates were quite variable under both light regimes, visual examination of Fig. 3 reveals greater distribution of flies in end trays (1 and 7 on the front axis) under light regime 2.

Fig. 3. Surface plots of fly attack rates in space (distance from emergence end, 4 = center of attack box) and time in a laboratory rearing system. Plots are turned so that early attack rates (time category 1, 0900-1030) are in the rear.

Conditions in facilities that rear phorid flies and other insects are likely to vary (e.g., lighting conditions outside of attack boxes may influence light intensity within), and subtle differences between individual attack boxes could influence fly distribution. These data indicate that phorid fly distribution can be manipulated by changing light intensity. Rearing systems should be evaluated in a similar manner to insure efficient use of rearing space and resources.

Acknowledgments

John Davis and Tim Swaggart (USDA-ARS-BCMRRU) assisted with collection of data. Dan Harsh was instrumental in constructing and testing attack boxes. Sanford Porter (USDA-ARS-CMAVE) provided starter culture of *P. curvatus*, advice on their requirements, and general rearing procedures. Debbie Boykin (USDA-ARS-MSA) provided statistical advice.
References Cited


MECHANISMS OF INTERCOLONY TRANSMISSION OF *THELOHANIA SOLENOPSIS* IN RED IMPORTED FIRE ANTS

David H. Oi & David F. Williams

USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology
1600 SW 23rd Drive, Gainesville, Florida 32608

*Thelohania solenopsae* is a microsporidian pathogen of imported fire ants. Infected queens have lower oviposition rates and die prematurely (Knell et al. 1977, Williams et al. 1999). The pathogen is transovarially transmitted but the natural mechanism of intercolony transmission is unknown. Infections of *T. solenopsae* can be initiated artificially in uninfected red imported fire ant, *Solenopsis invicta*, colonies by introducing live, infected brood (Williams et al. 1999, Oi et al. 2001). Because infected brood can be used for inoculations, we hypothesized that natural mechanisms of brood transfer between colonies can result in intercolony transmission.

Polygyne imported fire ants are not territorial and presumably share brood among colonies. Thus, it was hypothesized that *T. solenopsae* infected brood could be moved to uninfected colonies and serve as inoculum for new infections. Inoculations of *T. solenopsae* in ten southern U.S states in 1998–2000 resulted in infections in 9 of 15 inoculation sites. However, sustained infections, which also spread, occurred only in polygyne *S. invicta* populations. Infections in monogyne colonies generally were not found after initial detection except in Florida where infection has been detected for 3 years, but with little spread. Thus, there is circumstantial evidence that the movement of infected brood among polygynous colonies facilitates infection and spread.

For monogynous imported fire ant populations, we hypothesized that brood raiding may be a mechanism of horizontally transmitting *T. solenopsae*. Tschinkel (1992) described how incipient monogynous *S. invicta* colonies would steal brood from other incipient colonies. In support of our hypothesis, we have documented infection rates of 93 and 75% from male and female adult dates, respectively. Infection rates were obtained from 45 males from 7 colonies and 133 females from 9 colonies that were trapped as they took flight from nests during the initiation of mating flights. In addition, 30 newly mated queens that produced infected colonies have been collected and reared in the laboratory from 1999 to 2001. Average lifespan of these queens was 127 days ranging from 33 to 652 days (n=25) and colonies contained all life stages (i.e. eggs, larvae, pupae, and adults). Five of the 30 queens are still alive after a range of 239 to 1,012 days. Thus, it is evident that *T. solenopsae* infected incipient colonies can be produced.

To determine if imported fire ant colonies can become infected with *T. solenopsae* via brood raiding, 7 pairs of *S. invicta* colonies consisting of a large, uninfected and a small, infected colony were given access to each other in the laboratory. Large colonies contained an average of 5,571 (+1,134 SD) adults, 25.7 (+45 SD) ml of brood and 1 queen while small colonies contained 1,100 (+412) adults, 6.3 (+3.9) ml of brood and 1 queen. Small colonies had an infection rate of 30% based on a sample of 10 individual larval or prepupal smears. Queens from each colony were marked with paint to distinguish which colony they were from. In addition, 7 pairs of uninfected large and small colonies were used as controls.

To determine if imported fire ant colonies can become infected with *T. solenopsae* via brood raiding, 7 pairs of *S. invicta* colonies consisting of a large, uninfected and a small, infected colony were given access to each other in the laboratory. Large colonies contained an average of 5,571 (+1,134 SD) adults, 25.7 (+45 SD) ml of brood and 1 queen while small colonies contained 1,100 (+412) adults, 6.3 (+3.9) ml of brood and 1 queen. Small colonies had an infection rate of 30% based on a sample of 10 individual larval or prepupal smears. Queens from each colony were marked with paint to distinguish which colony they were from. In addition, 7 pairs of uninfected large and small colonies were used as controls.
After an average of 8.8 days (±4.9 SD) all brood was found within a single colony in 6 of the 7 pairs and 5 of 7 of the queens from the small, infected colonies died. *T. solenopsae* infection was detected in 4 of 7 of the large colonies. Average maximum infection rate for these colonies was 80% (±34 SD). For the controls all brood was found within a single colony after 6.5 days (±2.3 SD) in 6 of 7 pairs and 6 of 7 queens from the small colonies died. *T. solenopsae* was not detected in any of the control colonies. In the large colonies where *T. solenopsae* was detected brood levels declined an average of 69% after 22 weeks in contrast to an 88% increase in the controls. This further indicated that *T. solenopsae* infection had established and was impacting the fire ant colonies inoculated through brood raiding. In summary, sharing of infected brood and brood raiding are two ways *T. solenopsae* can be transmitted between colonies of red imported fire ants.

References Cited


YELLOW HEAD DISEASE (YHD) CAUSED BY A NEWLY DISCOVERED Mattesia SP. IN POPULATIONS OF THE RED IMPORTED FIRE ANT, Solenopsis invicta

Roberto M. Pereira, David F. Williams, James J. Becnel & David H. Qi

USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology

Surveys of fire ant pathogens conducted both in the imported fire ant homeland in South America (Jouvenaz et al. 1980) and in the United States (Jouvenaz et al. 1977, Beckham et al. 1982) have revealed few pathogens that can be used in biological control programs. Most of these pathogens including some Neogregarines. The Neogregarine Mattesia geminata infects larvae of Solenopsis geminata and causes mortality during the pupal stage (Jouvenaz & Anthony 1979). Infected S. geminata pupae turn black before dying, and infection does not occur in the adult ant. Mattesia geminata also infects several other Myrmicininae ants, and dying adult insects have been observed to harbor oocysts of the pathogen (Buschinger & Kleespies 1999).

Recent surveys undertaken in Florida revealed a new protozoan in S. invicta populations, occurring in workers and female reproductives. The disease has not been observed in immature S. invicta. Heads, and sometimes thoraxes, of infected workers and female reproductives show an atypical yellow-orange color. We have designated this disease as Yellow Head Disease (YHD) due to this distinctive characteristic. Many spindle-shaped oocysts can be observed inside the body of infected ants. Oocysts can be seen through the cuticle in all body regions but are easily recognized in the head and the appendages. Oocysts occur in pairs typical of Mattesia species (Weiser 1955). Ants with the typical yellow-head coloration may contain no oocysts but only bilobed structures similar to developing gametocysts with presporal stages of M. geminata described by Kleespies et al. (1997). These structures have led to the tentative classification of this pathogen in the genus Mattesia.

Oocysts are 18.7 ± 0.80 μm (mean ± sem; n=50) long and 10.3 ± 0.80 μm wide, therefore larger than oocysts of M. geminata (13-14 x 8-9 μm; Buschinger et al. 1995, Kleespies et al. 1997), M. trogodermae (11-13 pm; Hall et al. 1971), M. dispora (15.4 μm; Zizka 1978), M. poudryi (1 1 μm; Weiser 1952), M. grandis (11.8 μm; McLaughlin 1965) and Mattesia sp. (11.2 μm; Wright 1993). Oocysts were shorter and wider than those of M. bombi (21.6-27 μm by 5.4 μm; Liu et al. 1974). The oocysts of the YHD-causing agent have the length-to-width ratio at 1.83 ± 0.155 (n=50) compared to 1.44-1.48 for M. geminata (Buschinger et al. 1995, Kleespies et al. 1997). Distinct characteristics of oocysts, presence of oocysts in adult ants, and the yellow-head sign are evidence that YHD and its causative agent are new discoveries without established taxonomic identification.

Sixty-four central and northern Florida sites and 1017 fire ant nests were examined for the presence of the Mattesia-like oocysts or the presence of "yellow-heads". At each site, we sampled ants from 1-81 mounds using a 7-ml plastic vial coated internally with Fluon®. Occasionally, whole colonies were collected in 20-liter buckets. In the laboratory, we examined whole ants or ant macerates under a phase-contrast microscopy (200-630X) for the mature oocysts or bilobed presporal form.

The YHD is widely distributed in Florida, affecting both polygynus and monogyne S. invicta colonies. The disease was present in 34% of the sites and in 8% of nests sampled. In infected sites, the pathogen was observed in 19% of the nests. Dual infection with YHD and T. solenopsae occurred in at least one colony and in individual ants in this colony. Field colonies
brought into the laboratory had large mortality of YHD-infected ants within days after arrival. For instance, a 250-mg live ant sample from a field-collected colony had only 3.6% infected ants whereas accumulated cadavers collected a week later from the same colony had 49% of individuals with oocysts or bilobed gametocysts. This rapid mortality of infected ants potentially indicates that this disease may have significant impact on fire ant populations.

The observed wide distribution of the previously undetected YHD is an indication that the causative agent may have dispersed through the fire ant population very rapidly. High dispersal potential is a desirable characteristic for biological control agent. This new microbial pathogen from *S. invicta* colonies is distinct from any described disease, and may have significant impact on fire ant populations.

REFERENCES


A NEW TECHNIQUE FOR LABORATORY ASSESSMENT OF RED IMPORTED FIRE ANT MOUND DRENCH TREATMENTS

Bastiaan M. Drees
Department of Entomology, Texas A&M University, College Station, Texas

ABSTRACT

A new laboratory technique is described for assessing candidate liquid mound drench treatments against the red imported fire ant using inexpensive, readily-available equipment. The method utilizes cotton swab sticks to provide a walking surface for ants enclosed in soda straws pinched at both ends using clips to provide an encapsulation which can be submerged in candidate liquids. The method was used to assess contact insecticide properties of treatments on worker ant samples. Treatments evaluated included various dilutions of liquid dishwashing detergent and plant oil (citrus oil containing d-limonene) and plant oil-containing products as follows: Garden-Ville Soil Conditioner (30% orange oil plus 70% liquid compost), Citrex™ (78.2% d-limonene), Concern® (5.8% d-limonene), Exxant (14.2% terpentine plus 0.2% ammonia) and TFA Super-Kill™ (89% pine oil). Data generated using this technique can help in the rapid development of ant mound drench treatments prior to labor-intensive and costly field trials. This method is also suitable for assessing effects of variables such as exposure time, concentration, and temperature of solutions on efficacy.

This manuscript has been accepted in the Southwestern Entomologist. The complete article will be printed in an upcoming issue.
FIPRONIL GRANULAR AND BAIT + SEVIN® TWO-STEP TREATMENTS AND THEIR EFFECTS ON FIRE ANT “PAVEMENT” MOUNDS

Charles L. Barr, Texas Cooperative Extension

Abstract

In 2001, Aventis Environmental Science sponsored a trial to study the effectiveness of products containing the active ingredient fipronil in controlling colonies of red imported fire ants (Solenopsis invicta Buren). Treatments included four replications of fipronil formulated as a 0.0143% granule and a 0.0005% bait on corn cob grit, and a standard treatments of Amdro® (0.073% hydramethylnon) all applied broadcast. All three treatments were duplicated and included a follow up “two-step” treatment of Sevin SL (43% carbaryl). Also included as a standard was broadcast granular Talstar® (0.2% bifenthrin) and Sevin SL alone as an individual mound drench. The test was applied in October, 2001 at the Hallettsville Municipal Airport in south central Texas. The site also offered the opportunity to test these products’ effectiveness against “pavement” mounds - colonies adjacent to or under asphalt, concrete or buildings - where control is often difficult. Preliminary results through four months post-treatment included several findings. All broadcast treatments reached maximum suppression at about four weeks, except Talstar at two weeks. The application of Sevin mound treatments at 10 days post-treatment sped the activity of the broadcast products by only about two weeks at considerable expense in terms of product and labor. Mound treatments alone gave rapid control, but gave poorer overall control as previously untreated mounds appeared after rainfall. Control of mounds away from pavement by broadcast or two-step treatments ranged from 80% for Amdro alone to 100% for Talstar, with the others yielding 93-96% control. Pavement mounds were controlled most rapidly and effectively by Talstar, most slowly by granular fipronil. Mound treatments produced the worst control of pavement mounds at only about 60% reduction compared to untreated plots. Maximum control of pavement mounds was 100% by Talstar, 97% by granular fipronil. The test raises the question of why a fast-acting contact insecticide, Talstar, produced 100% control of pavement mounds while baits and slower-acting products did not, contrary to conventional wisdom. This test is ongoing. Full results will be reported through Texas Cooperative Extension upon its completion in the fall of 2002.
AREAWIDE SUPPRESSION OF FIRE ANT POPULATIONS IN PASTURES: PROJECT OVERVIEW

Roberto M. Pereira

USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology

The red imported fire ant was accidentally introduced into the United States without most of the biological control organisms that kill fire ants in their native South America. Because of their potent sting and large populations, fire ants cause serious medical and agricultural problems to people, animals, and equipment. Damage to pastures is especially difficult to manage because fire ants are expensive to control over the large acreage. Fire ants also have a severe ecological impact on ground-nesting birds and mammals, predators and parasites of pests, and other ants. Over the past decades, chemical baits and other products have been the only means to control fire ants. However, with the successful establishment of biocontrol agents in several US locations, an integrated approach to fire ant control is now possible.

In 2001, the USDA funded a 5-year project on areawide control of fire ants with an overall goal of maintaining low fire ant populations with a reduced need for bait toxicants. This goal can be accomplished by using available self-sustaining fire ant biological control agents in conjunction with the bait toxicants. This project aims to demonstrate practical, long-term control of fire ants over a large area using an integrated approach. Demonstration sites for this project will be selected in Florida, Mississippi, Oklahoma, South Carolina, and Texas to represent the different environments where fire ants occur in the U.S.

The protocol for the project on Areawide Suppression of Fire Ant Populations in Pastures was approved by the Technical Core Committee which includes the following members: Robert Faust (USDA-ARS, Maryland) David Williams, David Oi, Sanford Porter, Robert Vander Meer, Roberto Pereira (USDA-ARS, Florida); Doug Streett, James Vogt (USDA-ARS, Mississippi), Anne-Marie Callcott (USDA-APHIS, Mississippi), Phillip Koehler (University of Florida); Russell Wright, Wayne Smith (Oklahoma State University); Mac Horton, Tim Davis (Clemson University, South Carolina); Bastiaan Drees, and Charles Barr, (Texas AM University).

In each state, 2 sites will be established in improved pastures, one in which the biological control agents will be established and a control site with no biocontrols. In MS, 2 sites will be established in an area infested with hybrid fire ants and 2 sites in black fire ant region. Each site will contain a 300-acre central area that will be treated with chemical fire ant baits and some peripheral acreage where biologicals will or will not be established. Application of the biocontrol agents in the peripheral area will serve to prevent, limit, or slow reinfestation of the chemically treated area. These biocontrol agents will be introduced using the inoculative approach that has been successful in other areas.
Biological controls to be used in this demonstration project are the microsporidium *Thelohania solenopsae* and the parasitic decapitating flies of the genus *Pseudacteon*. The phorid flies will be released at test sites over a 2-week period near disturbed fire ant mounds. *Thelohania solenopsae* will be added to active fire ant mounds as live, *T. solenopsae*-infected brood, (3 g/mound). The chemical bait used in this project is a 1:1 mixture of products containing hydramethylnon ("Amdro" or "Siege") and methoprene ("Extinguish"), used at a rate of 1.5 lbs/acre.

In the treated area, 20 1/8-acre circular plots will be established in high fire ant density areas, whereas 30 circular plots will be established in the peripheral areas. These plots will be used in monitoring fire ant activity (using hot dog baits), fire ant population (using the USDA population index and mound counts), and biodiversity (using pitfall traps). Presence of Phorid flies and *T. solenopsae* will also be monitored to determine the rate of spread of these biocontrol organisms over the experimental areas.

This project also contains economic and educational components. The economic component aims to examine the economic visibility of the integrated approach to fire ant control and cost/benefit impact of the proposed project. This analysis will look at the impact of fire ant infestations at the farm level, as well as project overall economic impact in cooperating states and US. The educational component will involve the preparation of informational material for distribution to the public, educational institutions, media outlets, etc. A brochure has been prepared explaining the project and was distributed through cattlemen's magazines and associations. A webpage (http://fireant.ifas.ufl.edu) is currently active and will be updated with new features, including video clips showing ant activities and biocontrol agents.

This areawide project is expected to generate several outcome such as: a) release and spread of biological controls, b) sustained fire ant control, c) lower livestock production costs, d) increased farmworker safety, e) reduced pesticide risk, f) restored ecological balance among native ants, birds, and wildlife, g) demonstration of economic benefits of fire ant management in large areas, and h) increased public knowledge and awareness of new technologies and management tools for fire ant control.
AN UPDATE OF THE RED IMPORTED FIRE ANT PROGRAM FOR THE CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE

Mohammad Azhar

California Department of Food and Agriculture
Red Imported Fire Ant Program
PO Box 11239 Costa Mesa, CA 92627
MAzh@cdfa.ca.gov

Abstract

Initial infestations of Red Imported Fire Ant (RIFA) [Solenopsis invicta (Buren)] in the Central Valley were detected in 1997 and were traced back to beehives brought from infested southern states for almond orchard pollination. California Department of Food and Agriculture (CDFA) maintains a dual approach of preventing the entry and spread of RIFA in California through border station inspections, pest interceptions, and through early detection within the state. Following the detection of RIFA in nurseries, landscaped areas, parks, etc in Orange, Riverside, and Los Angeles counties in 1998, CDFA established quarantines in the entire county of Orange and parts of Riverside and Los Angeles Counties. The quarantine was designed to contain RIFA spread by requiring inspection and treatment of articles thought to spread RIFA such as nursery stocks, soil, sod/turf grass, hay, landscaping, and bee colonies.

The Secretary of Agriculture based on recommendations of Science Advisory Panel proposed RIFA Action Plan in March 1999. RIFA Control/Eradication Program was set up by CDFA to control the spread and eradicate known infested sites. The Program, in collaboration with California County Agricultural Commissioners and other agencies cooperatively, survey, treat, and regulate the movement of regulated articles both within and out of the quarantined areas, through inspections, compliance agreements, and certifications. There had been no major RIFA infestations in the state since the quarantine was established. Local Agencies are playing a major role in detection, eradication and public outreach in urban areas. Department of Pesticide Regulation and University of California researchers along with other agencies do environmental monitoring of pesticide use and research.
FIRE ANTS DOWN-UNDER: PROGRESS TO DATE ON THE AUSTRALIAN NATIONAL ERADICATION PLAN FOR SOLENOPSIS INVICTA

Cas Vanderwoude
Keith McCubbin
Queensland Department of Primary Industries, Fire Ant Control Centre, P.O. box 1241, Oxley, Queensland, Australia 4075

Abstract—Red Imported Fire Ants have been discovered in Brisbane Australia spanning over 37,000 hectares of urban and industrial suburbs. In a little more than 12 months, a national response has been formulated which includes a program to eradicate fire ants from known locations. To date, the infested area has been treated three times with corn/oil baits containing insect growth regulators and a large area of land surrounding the treatment zone has been inspected to ensure no nests have been missed. Preliminary monitoring results indicate a high level of control is being achieved.

History of Detection—Brisbane is the state capital of Queensland and is located on Australia’s eastern coast-line (Figure 1). The climate is sub-tropical; average rainfall is approximately 1200 mm per annum (summer maximum) and frosts are rare. In short, Brisbane’s climate is ideal for Solenopsis invicta.

Red Imported Fire Ants were discovered in Brisbane in February 2001 as a result of specimens submitted for identification to the Queensland Museum and the Queensland Department of Primary Industries (Nattrass & Vanderwoude 2001). These identifications were subsequently confirmed by a taxonomist at the CSIRO Australian National Insect Collection. Knowledge of impacts of Red Imported Fire Ants on agriculture (see (Glancey et al. 1979; Lofgren & Adams 1981; Smittle et al. 1983; Lofgren 1986; Vinson & Sorensen 1986; Stewart & Vinson 1991; Drees et al. 1998); human health (James et al. 1976; Adams & Lofgren 1982; deShazo et al. 1984; Stablein et al. 1985; Bahna et al. 1988; Rhoades et al. 1988; Swanson & Leveque 1990; Stafford 1996; Levy et al. 1998; Caldwell et al. 1999); and the environment (Porter & Savignano 1990; Allen et al. 1995; Giuliano et al. 1996; Pedersen et al. 1996; Allen et al. 1997; Holtcamp et al. 1997; Mueller et al. 1999; Allen et al. 2001; Forys et al. 2001; Zettler et al. 2001) in the south-eastern part of the USA triggered an emergency response by the Department of Primary Industries.
Under national cost-sharing arrangements for exotic pest incursions, a scoping study commenced in March 2001 (completed June 2001) with the aim of determining the area infested; preventing further spread through restricting movement of high-risk items; assessing the effectiveness of various treatment options and evaluating the feasibility of eradicating Red Imported Fire Ants from Australia.

Extensive surveillance for Red Imported Fire Ants was carried out in Brisbane during this period. Over 9000 properties were inspected for Red Imported Fire Ants in these first three months alone. Almost 4000 inspections were as a result of requests by the public due largely to intense media interest. Surveillance activities quickly established that there were two loci: one near the mouth of the Brisbane River and the main shipping port; and another in the south-western suburbs some 20 miles apart. The total infested area covers approximately 37 000 ha (Figure 2).

Through excavation of nests and colony morpho-metrics, it appeared that the eastern locus consisted exclusively of monogyne colonies and the western locus exclusively of polygyne colonies. It now appears that the western population consists of a mixture of monogyne and polygyne colonies (R. Crozier & N. Kunzmann, James Cook University, unpublished data). Investigations since that time have also established a number of differences between the two populations leading to the probability that each locus was the result of a separate introduction. Evidence to support this notion includes:

1. Substantial variation (~5%) of mitochondrial DNA (J. Hughes, Griffith University; pers. comm.) between loci; and
2. Distinct differences in biochemistry suggesting the western population may have originated in northern Argentina while the eastern population originated elsewhere (R. Vander Meer, USDA Gainesville, pers. comm.).

![Figure 2. Map of Brisbane and surrounding cities. Areas infested with S. invicta are shaded](image)
Development of an eradication plan-A plan to eradicate Red Imported Fire Ants was developed by a scientific panel of myrmecologists and pest incursion specialists, operational managers and a team of specialists from the U.S.A. (S.D. Porter, C.L. Barr and B.M. Drees). The approach consists of the following key activities:

1. Repeated applications of corn/oil baits containing insect growth regulators (pyriproxyfen or S-methoprene) to the entire putatively-infested area over a three year period;
2. Intensive surveillance of areas immediately outside the infested area during the treatment phase;
3. State-wide passive surveillance coupled with and national surveillance of high risk areas (nurseries, transport depots shipping ports etc programs to ensure no infestations remain undetected; and
4. Intensive surveillance of the treated areas for two years following the cessation of treatment.

This program has a budget of approximately $AUS140 million over 5 years and is being implemented by the Fire Ant Control Centre with a workforce of approximately 500 employees. The majority of personnel and budget are dedicated to the task of treatment or surveillance. However, there are several support programs to these core activities including public relations and community engagement; movement controls and industry liaison; GIS and database; and scientific services.

The research team consists of four main projects. There is a Diagnostics section that processes samples submitted by surveillance teams and members of the public. A Research and Development section deals with applied research of new treatment options and other operational issues. The Ecology and Environment unit researches Red Imported Fire Ant biology and ecology in Australia; and works with several universities and postgraduate students to assess the impact of broad-acre treatment on non-target fauna. A monitoring project assesses the control levels achieved by the treatment program.

Progress to date-It has been a little over 12 months since the initial discovery of Red Imported Fire Ants in Brisbane. That time has seen the formation an eradication plan, establishment of the Fire Ant Control Centre, and the application of several rounds of treatment (Table 1).

Table 1. Timeline of key events since the detection of Red Imported Fire Ants in Brisbane

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Jan 2001</td>
<td>suspect ant specimen submitted</td>
</tr>
<tr>
<td>22 Feb 2001</td>
<td>positive identification</td>
</tr>
<tr>
<td>26 Feb 2001</td>
<td>emergence response unit formed</td>
</tr>
<tr>
<td>19 June 2001</td>
<td>scoping study complete</td>
</tr>
<tr>
<td></td>
<td>5 year plan and budgets finalised</td>
</tr>
<tr>
<td>3 Aug 2001</td>
<td>funding approved &amp; program launched</td>
</tr>
<tr>
<td>24 Sept 2001</td>
<td>&gt;500 staff hired and trained</td>
</tr>
<tr>
<td></td>
<td>treatment commences</td>
</tr>
<tr>
<td>18 March 2002</td>
<td>third round of treatments applied</td>
</tr>
<tr>
<td></td>
<td>movement control legislation in place</td>
</tr>
</tbody>
</table>
In order to measure the efficacy of treatment, approximately 30 plots were established in known infested areas. At each site, the plot boundaries were marked with pegs and all visible nests within and immediately adjacent to the plot boundaries were marked with pink tags. The location of each nest was mapped using a 50 cm grid of the site. Plots mapped at establishment and re-mapped in early January. The relative aggression of workers in each nest was measured on a four point scale in response to repeated penetration of the nest by a steel wire to a depth of 100 mm where 0 = inactive, 1 = 1-5 ants, 2 = 6-50 ants and 3 = >50 ants. Mounds with no activity were considered “dead”.

Pitfall traps were established in permanent sleeves in a layout consistent with plot shape. Depending on site, a minimum of 10 pitfalls at 4 metre intervals was established. Pitfalls were opened monthly for 72 hours. Ants were separated from other pitfall trap material and sorted to genus. Numerical counts of each ant genus were recorded unless abundance of an ant genus in a pitfall exceeded 500 (native *Solenopsis* were not included with *S. invicta* counts).

When all data were combined, over 80% of Red Imported Fire Ant mounds in monitoring plots were inactive when assessed in January and less than 7% displayed high levels of response to nest disturbance. When results were stratified by nest density, greater control was achieved in plots with high (>500 mounds/ha) and moderate (101-500 nests/ha) numbers of mounds, while lower control was achieved in low density plots (Figure 3). Sorting and analysis of pitfall trapping on monitoring sites is not complete. Data from 15 sites have been processed. Ant abundance (mean number of ants per pitfall) reduced by 84% (s.e. ±6.21) between September and January.

Figure 3. Mean levels of control and vigour of remaining colonies for densely infested, moderately infested and lightly infested monitoring locations.
Discussion—Mound counts and pitfall trapping both indicated a high level of control for Red Imported Fire Ants with control rates exceeding the 70% target. We acknowledge that these results are preliminary in nature as the active ingredients in the baits are slow-acting and results difficult to assess in a short time-frame. However, the effects at some sites has been nothing less than spectacular. At one notable location, an infestation of 163 active mounds in a single back yard of 336 m² (>5100 mounds/ha) was reduced to just four active colonies. Not all treated sites were this successful. For example, at another high-density infestation, the density was reduced from 1600 mounds/ha to 740 mounds/ha indicating some variability in treatment efficacy.

Highest variability was recorded at low density infestations where a mean control rate of 44% was achieved. This is substantially lower than the >80% achieved at densely infested areas (although preliminary analysis of end-season data indicates control is much higher). Many other ant species are present at low-density sites and there is strong competition for resources. At these sites, Red Imported Fire Ants are rarely dominant on the soil surface. Therefore, it is possible that much of the initial application of bait was consumed by native ants. We predict that this is likely to reduce competition for the bait by native ants for future treatments and that control rates for Red Imported Fire Ants will increase with subsequent treatments.

References


STUDIES ON ATTRACTIVENESS AND EFFECTIVENESS OF AN ARTIFICIAL ENTOMOPHAGE DIET FED TO HYBRID IMPORTED FIRE ANTS

James T. Vogt and Allen C. Cohen

USDA-ARS-BCMRRU
P. O. Box 225
Stoneville, Mississippi 38776

USDA-ARS-BCMRRU
Box 5367
Mississippi State, MS 39762

Abstract

An artificial entomophage diet (Cohen, U. S. Patent # 5,834,177. November 10, 1998) was offered to Solenopsis invicta Buren x Solenopsis richteri Forel (hybrid imported fire ant) in a series of choice tests. Foraging workers collected ≈ 27 times more reconstituted diet than freeze-dried diet, and collected similar amounts of reconstituted diet and freeze-killed, macerated cricket. Even though workers were strongly attracted to the artificial diet, all measures of colony growth (mean mass of brood, workers, and queen) were at least 50% lower in colonies fed sugar water + artificial diet than in colonies fed sugar water + crickets or sugar water + artificial diet + crickets. While this highly attractive diet may have some utility as a bait for monitoring fire ants in the field, feeding colonies sugar water and the diet alone or in combination with crickets offers no advantage over a diet of crickets and sugar water.

Introduction

Various diets have been proposed for rearing imported fire ants (Khan et al. 1967, Bhatkar and Whitcomb 1970, Banks et al. 1981, Porter 1989); however, none have proven satisfactory without whole insects, offered separately or in the diet. Porter (1989) observed no difference in growth of imported fire ant colonies fed sugar water + artificial diet + crickets and sugar water + an artificial diet + crickets.

An artificial diet for rearing imported fire ants would offer many benefits, either as a standalone diet or bulk supplement. A standalone diet could be manipulated for precise nutritional studies. A bulk supplement might be useful for increasing production in laboratory colonies, an important consideration for mass-rearing of disease organisms and parasitoids associated with imported fire ants. We tested an artificial diet (Cohen, U. S. Patent # 5,834,177. November 10, 1998) for palatability and effectiveness for rearing hybrid imported fire ants (Solenopsis invicta Buren x Solenopsis richteri Forel) in the laboratory. The diet was designed for rearing green lacewings, Chrysoperla spp. (Neuroptera: Chrysopidae), and has proven satisfactory for mass-rearing multiple generations of these entomophages (Cohen and Smith 1998).
Previously, a series of choice tests was used to determine attractiveness of the diet (freeze dried and reconstituted) vs. a freeze-killed, macerated cricket (*Acheta domestica* L.) standard. Foraging hybrid imported fire ants were highly attracted to reconstituted diet, and collected equal amounts of cricket and reconstituted diet. In a small-scale field trial, hybrid imported fire ants were attracted to reconstituted diet in large numbers. This work is currently in review for publication and will not be dealt with here.

**Materials and Methods**

An experiment was designed to measure colony growth of hybrid fire ant colonies receiving sugar water + crickets, sugar water + artificial diet, and sugar water + crickets + artificial diet. Colonies (*n=15*) were collected from the field (Oktibbeha Co., MS, USA) and standardized just prior to beginning the experiment. Each standard colony contained 1 physogastric queen, 5 g workers, and 2 g brood. Colonies were housed in trays (41.75 cm L X 27.5 cm W X 12 cm H) and provided a castone® nest (150 mm X 25 mm), water, and 1 M sugar solution. Crickets and artificial diet were offered separately in 1 oz. plastic soufflé cups, with small (0.3 mm) holes drilled in the side for forager access, and lids to slow desiccation of the contents. Cups were checked daily for mold and desiccation, and colonies were provided the appropriate foods *ad libitum* for a total of 8 wk. The experimental design was a completely randomized design replicated 5 times, and data were analyzed using Proc Mixed (Littell et al. 1996) followed by Least Squares Means to test for treatment effects on queen mass, total brood mass, total worker mass, and total colony mass. Means are reported as mean ± SE.

**Results and Discussion**

All measures of colony fitness and/or growth were significantly lower in colonies fed sugar water + artificial diet than colonies fed sugar water + crickets and sugar water + artificial diet + crickets (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Queen mass (mg)</th>
<th>Brood mass (mg)</th>
<th>Worker mass (mg)</th>
<th>Total mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar water + crickets</td>
<td>20.0 ± 2.0a</td>
<td>24.0 ± 5.1a</td>
<td>15.1 ± 3.0a</td>
<td>39.1 ± 7.8a</td>
</tr>
<tr>
<td>Sugar water + artificial diet</td>
<td>14.1 ± 1.3b</td>
<td>4.3 ± 4.3b</td>
<td>5.6 ± 3.7b</td>
<td>9.9 ± 7.9b</td>
</tr>
<tr>
<td>Sugar water + crickets + artificial diet</td>
<td>24.1 ± 1.3a</td>
<td>23.2 ± 3.9a</td>
<td>10.6 ± 2.2ab</td>
<td>33.7 ± 5.8a</td>
</tr>
</tbody>
</table>

1 Means in a column followed by the same letter are not significantly different (Least Squares Means, *P* > 0.05).

Unfortunately, while it may have utility as a bait for monitoring imported fire ants in the field, the artificial diet offers no advantage alone or in combination with a standard diet of crickets for rearing fire ant colonies. Interestingly, growth of colonies fed sugar water + artificial diet appeared to keep pace with growth in other treatments until ≈ 4 wk into the experiment, but data were only collected once at 8 wk. All colonies offered artificial diet collected and appeared to store large amounts of the diet, but we did not measure actual consumption. Reduction in queen mass for colonies fed sugar water + artificial diet might suggest some nutrient deficiency.
affecting reproductive capacity. A close examination of micronutrient content of the diet and crickets may provide direction for improvement.

Acknowledgments

We thank J. R. Davis (USDA-ARS, BCMRRU) for expert technical assistance.

References Cited


ANT FAUNA OF CANOPIED AND OPEN HABITATS IN CENTRAL GEORGIA

Reid Ipser, Hal Peeler, and Wayne Gardner

Department of Entomology, College of Agricultural and Environmental Sciences, 1109 Experiment Street University of Georgia, Griffin, GA 30223

Introduction. Knowledge of species composition, community structure, and specific ant assemblages can be used in several ways. For example, in Australia, ants are one of the most functionally important faunal groups (Matthews and Kitching 1984, Anderson 1992) and are model organisms for studies in community ecology (Anderson 1983, 1988, 1991, Greenslade and Halliday 1983). Ants also have been used as bio-indicators in mine-site rehabilitation (Majer 1983, 1985).

A large diversity of interactions occur between ants and other organisms. Schultz and McGlynn (2000) indicate that if such interactions are properly understood, one could predict ecological conditions within a given habitat based upon the presence of a particular ant species. In addition, one could establish empirical correlations of the presence of a particular ant species with specific ecological conditions. Moreover, these correlations can be used as predictors of ant interactions and biodiversity, especially if one focuses on ant species that participate in precise, obligate interactions with other ant species (Schultz and McGlynn 2000).

Procedures. The study was conducted at two state parks located in south-central Georgia (i.e., High Falls, Indian Springs). Each of these parks contains more than 200 ha of wooded areas characterized as second-growth forests, comprised of oak, loblolly pine, slash pine, and other softwood and hardwood species. Each also includes over 100 ha of open areas. Sampling sites in the second growth-forests were located 60 m from right-of-way or open roads and encompassed an area of 1000 m² each. Adjacent open fields, characterized as frequently disturbed fields (mowed approximately once a month) containing primarily grasses, were used as comparison sample sites. The area of each open field sampled also encompassed 1000 m². Each site was sampled once a month.

Ground-dwelling ant fauna were sampled using pitfall traps, bait traps, collection of leaf litter, and visual searching. Bestlemeeyer et al. (2000) cite that these techniques yield the best results for total species richness, epigaeic ants, forager abundance, and population levels.

Twenty pitfall traps were placed at 1-m intervals along each transect. The traps were 40-ml plastic vials containing propylene glycol (filled 2/3) as a nontoxic preservative. They were inserted into the ground to a depth such that the upper rim of the vial was level with the soil surface. After 7 days, each trap was removed, capped, and returned to the laboratory.

Petri dishes (35 x 100 mm) containing 1 g of canned tuna fish in oil were used as bait traps. Dishes were placed at 2-m intervals along the same transects. Dishes were left exposed for 2 h. They were then covered, sealed, and transported to the laboratory.
Litter samples were collected at 5-m intervals along each transect. This involved hand collecting litter and humus in a 1-m² area and placing the collected samples in large trash bags. Collected litter was transported to the laboratory where sub-samples were placed into Berlese funnels. After 48 h, vials containing ants and other invertebrates were removed and separated for ants.

Each sampling site was also visually searched for ant fauna for 2 man-hours. Litter, bare ground, tree trunks, foliage, decaying wood, etc. were searched. Representative ants were collected and placed in 70% ETOH for transport to the laboratory.

All ants collected by these methods were initially identified by comparison with specimens deposited in the UGA Natural History Museum (Athens, GA). These identifications were verified by Mark Deyrup (Archbold research station, Lake Placid, Fl.)

Results and Discussion. Species collected from September through December 2001 are listed in Table 1. Among the species collected, two appear to be new distribution records for Georgia. These are *Stenamma favolocephalum* and *Stenamma schmitti*, which we collected from both sites. *Prenolepis imparis* was the most numerous ant collected from canopied areas with 7,635 specimens collected over a 4-month period. An additional 45 were collected from un-canopied (open) areas. Two other native ants, *Pheidole tysoni* (2,197) and *Monomorium minimum* (1,303) were the most numerous in un-canopied areas (table 2). Preliminary observations indicate that *Prenolepis imparis, Pheidole tysoni, and Monomorium minimum* exhibit priority effects at baits, preventing *Solenopsis invicta* from monopolizing baits. However, more *S. invicta* were collected in the un-canopied areas than in the canopied areas, with 6.5x more collected from un-canopied than from canopied areas in High Falls Park and 200x more collected from un-canopied than canopied areas in Indian Springs Park.

References Cited


Table 1. Species list for High Falls and Indian Springs State Parks

| Aphaenogaster fulva          | Paratrechina vividula              |
| Aphaenogaster mimiana       | Paratrechina faisonensis           |
| Aphaenogaster lamellidens   | Pheidole bicarinata vinelandica    |
| Aphaenogaster picea         | Pheidole crassicornis              |
| Aphaenogaster picea rudis   | Pheidole dentata                   |
| Aphaenogaster texana        | Pheidole dentigula                 |
| Aphaenogaster texana carolinensis | Pheidole tysoni              |
| Brachymyrmex depilis        | Ponera pennsylvanica               |
| Brachymyrmex musculus       | Prenolepis impairs                 |
| Camponotus americanus       | Pyramica rostrata                  |
| Camponotus pennsylvanicus   | Solenopsis invicta                 |
| Camponotus lineolata        | Solenopsis molesta                 |
| Formica pallidefulva        | Solenopsis carolinensis            |
| Formica schaufussi          | Solenopsis texana                  |
| Formica subsericea          | Solenopsis truncorum               |
| Forelius mccooki            | Stenamma diecki                    |
| Hypoponera opaciceps        | Stenamma fovelocephalum            |
| Hypoponera opacior          | Stenamma Schmitti                  |
| Monomorium minimum          | Strumigenys louisianae             |
| Myrmica punctiventris       |                                    |
| Pachycondyla chinensis      |                                    |
SEED PREFERENCES OF THE RED IMPORTED FIRE ANT IN OKLAHOMA

James T. Vogt¹, Stanley A. Rice², and Steven A. Armstrong³

¹USDA, ARS Biological Control of Pests Research Unit, P.O. Box 67, Stoneville, MS 38776
²Department of Biological Sciences, Southeastern Oklahoma State University, Durant, OK 74701
³Army Corps of Engineers, Skiatook Lake Office, HC67 Box 135, Skiatook, OK 74070

Abstract

Seeds constitute a small but significant portion of the diet of red imported fire ants (RIFA). A study was conducted to compare relative attractiveness of seeds from several forbs used for roadside beautification in Oklahoma. In a preliminary study, foraging ants ignored dry seeds, so seeds were hydrated prior to use. RIFA in a field in southeastern Oklahoma preferred seeds of Echinacea purpurea (purple coneflower) more than those of Monarda citriodora (lemon mint), Rudbeckia hirta (black-eyed susan), and Gaillardia pulchella (Indian blanket); they showed a negligible interest in Coreopsis tinctoria (plains coreopsis), Rudbeckia amplexicaulis (clasping-leafed coneflower), Coreopsis lanceolata (lance coreopsis), and Lupinus texensis (Texas bluebonnet). Preferences of RIFA for consuming and/or dispersing different seeds may affect the relative species composition of natural areas and have an impact on the use of seedlings for reclamation.
HIGHER-ORDER PREDATION BY RED IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE) AND ITS IMPACT ON COTTON APHID (HOMOPTERA: APHIDIDAE) POPULATIONS

Ian Kaplan and Micky D. Eubanks

Department of Entomology and Plant Pathology, Auburn University

Red imported fire ants, Solenopsis invicta, are an invasive species found in high densities throughout the southeastern United States. Agricultural fields are particularly sensitive to fire ants due to their aggressive, predatory nature and the simplified insect fauna found in these systems. Fire ant presence in agricultural systems has been theorized to provide beneficial control of pest species. Alternatively, it has also been hypothesized that fire ants disrupt pest control through interference of natural enemies. In Southeastern cotton fields, fire ants may interfere with predators of cotton aphids, Aphis gossypii. Fire ants and cotton aphids may engage in a mutually beneficial relationship whereby fire ants protect aphids from natural enemies in exchange for honeydew. Aphid honeydew is a sugary solution produced by aphids that ants may use as food. In both caged greenhouse experiments and large-scale field experiments we tested the hypothesis that fire ants defend aphids from ladybird beetle larvae and green lacewing larvae, Chrysoperla carnea, and that this protection contributes to aphid epidemics. Ladybird beetle and green lacewing larvae were chosen because they are abundant predators that consume large numbers of aphids in Alabama cotton fields. Their consumption rates may regulate aphid populations below levels that are economically damaging. Therefore, fire ant interference may release aphids from these biological control agents. The purpose of this experiment was to document the impact of aphid protection by red imported fire ants on cotton aphid survival.

We performed caged greenhouse experiments to test our hypothesis. In choice experiments, fire ants more frequently foraged on cotton plants with aphids than on cotton plants without aphids (approximately 103 ants/plant with aphids; approximately 5 ants/plant without aphids). These data suggest that aphids attract fire ants into the canopy of cotton plants. In other caged experiments, aphids exposed to ladybird beetle or green lacewing larvae demonstrated a significantly higher rate of survival when simultaneously exposed to fire ants. Aphid populations were reduced by 45% in the presence of ladybird beetle larvae and 63% in the presence of green lacewing larvae. With the addition of fire ants to the aphid-predator treatments, aphid populations approximately doubled. Fire ant response to predator presence was similar between larval types, both types of predators were instantaneously attacked by multiple ants upon detection. Neither predator demonstrated an ability to endure fire ant exposure and subsequent attack, they both suffered significant mortality (approximately 96%). This strongly suggests that fire ants disrupt aphid predation by ladybird beetle and green lacewing larvae.

During the 2000 growing season we sampled aphids weekly in cotton fields at the E.V. Smith research station. We used three large fields that were planted with Stoneville BXN47 and one that was planted with Paymaster 1218 BG/RR. These fields were at least 20 hectares and separated by 1-2 kilometers. Two 1.2 hectare plots were established at opposite ends of each field. Plots were separated by at least 100 meters. Plots were divided into two treatments: high
fire ant density or low fire ant density. Treatments were assigned randomly and established using Amdro®, a commercially available fire ant bait that decreases fire ant abundance. Amdro was applied manually (2.2 kg/0.4 hectare) 2-3 times during the field season. This treatment was effective at reducing fire ant densities. In Amdro treated field plots, fire ant density was reduced by 72%. Aphid sampling consisted of visually searching the upper six leaves of a cotton plant and counting all visible aphids on the top and bottom of each leaf. We randomly selected 10 plants per plot to be visually searched. We found that aphids were significantly more abundant in cotton plots with high densities of fire ants than in cotton plots with experimentally suppressed densities of fire ants (Figure 1).

Fig. 1

Results from our greenhouse and field experiments suggest that fire ants promote aphid outbreaks by protecting them from predators. Aphid honeydew appears to be the stimulus for this interaction. Observational evidence and empirical data from greenhouse experiments indicate that fire ant presence alone does not have a negative impact on aphid populations. This suggests that fire ants found in the canopy of cotton plants are involved in honeydew retrieval. Aphids, therefore, may serve as a stimulus for ant presence in the canopy of cotton plants. Our data indicates that this may be detrimental to the biological control of aphid populations. Alternatively, fire ant presence on plant foliage has the potential to stimulate the biological control of other pest species through chance encounter. Pest insects in cotton, including caterpillars, stinkbugs and tarnished plant bugs, can cause great amounts of damage. Therefore the cost of inflated aphid populations needs to be weighed against the potential benefit of enhanced biological control of alternative pest species. In future studies we plan on examining the effect of this fire ant-cotton aphid interaction on herbivorous insects.
FIRE ANT DAMAGE TO TARGET LIFTERS AT A NATIONAL GUARD FIRING RANGE IN TEXAS

Michael E. Merchant and Margie Barton

Texas Cooperative Extension, Texas A&M University Research and Extension Center, 17360 Coit Road, Dallas, TX 75275-6599. m-merchant@tamu.edu

Abstract

Two granular insecticides were evaluated for the control of red imported fire ants, Solenopsis invicta, in soil surrounding pop-up targets on a National Guard firing range in Mineral Wells, Texas between 1998 and 2001. Both fipronil 0.1 G- and tefluthrin 1.5 G-treated plots had significantly fewer numbers of fire ant colonies compared to untreated controls at eight months after treatment; however, there were no significant differences among treatments at 12, 18 and 24 months after the applications were made.

Fire ants were observed causing damage to natural rubber and neoprene foam components of the pop-up target lifters. Nearly 100% of electrical outlet weatherstripping and 22% of the rubber boots on pop-up targets were affected by fire ants prior to treatment. Also, 87% of the target lifter electrical outlets had exposed wiring due to insulation damage apparently caused by fire ants. Such damage poses a significant risk of mechanical and electrical system failures, and electrical shorts could pose safety hazards to workers.

Introduction

The red imported fire ant, Solenopsis invicta, is a persistent pest of ground-based electrical equipment throughout the southern United States (MacKay et al., 1992, Slowik et al. 1997). Recently, several National Guard camps in Texas have reported problems with fire ants infesting and damaging firing range pop-up targets (Merchant, 1999). Camps Maxey (Lamar County, Texas) and Swift (Bastrop County, Texas) report 20-40% loss of functionality in electronic pop-up targets due to fire ant invasion. Costs of this damage are significant and have resulted in equipment loss and down-time for the target ranges. In 1998 the entire pistol range at Camp Bowie was shut down for rewiring due to fire ants, at a repair cost of $35,000 (Paul Powell, Adjutant General’s Department, Texas Army National Guard, personal communication, 1998).

Our objectives were to assess the causes of fire ant damage and to identify promising granular insecticide treatments that might reduce the impact of fire ants on ground-mounted machinery, such as pop-up target lifters.

Methods and Materials

The study was conducted at the Fort Wolters combat pistol range in Mineral Wells, Texas. The combat pistol range had a history of fire ant infestation and a total of 60 working pop-up target sites. The range was installed in approximately 1989 (personal correspondence, LtC Gary Huffman).
On 20 April, 1999, and approximately every six months thereafter, fire ant activity was estimated by counting all active fire ant mounds within a 2.43 m (8 ft) radius circle around each target lifter (Figure 1). Mound size was estimated according to a modified rating system based on the work of Harlan et al. (1981). Colonies observed at the site were generally small and rated as 1 (less than 100 ants, mound less than 10 cm diameter), 2 (100-1,000 ants, and usually 10-40 cm diameter mound), or 3 (1,000 - 10,000 ants, mound usually greater than 40 cm diameter).

In addition to mound counts, assessments were made of foraging fire ant worker abundance on 19 October, 1999, 31 May, 2000 and 06 October, 2000, respectively. Glass shell vials (1 dram capacity) were baited with two pellets of Seafood Purina® Tender Vittles® cat food, and placed on the soil adjacent to each pad site. After a 2-3 hr exposures, vials were collected, stoppered, transported to the laboratory, and frozen. Ants collected in the vials were later counted and identified to species.

Figure 1. Plot surrounding pop-up target marked for evaluation of mound densities (left), and close up view of pop-up target lifter showing outlets and ground-based lifter unit. Fort Wolters Army National Guard Base, Mineral Wells, Texas. Summer, 1999.

Soil treatments were applied to lifter sites on 19 October, 1999. Insecticide treatments were applied uniformly to the soil inside a 4.88 m (16 ft) square plot surrounding each lifter vault. Granular formulations of fipronil (Collins et al., 1999) and tefluthrin (Merchant 1998) were applied using a shaker made from a 620 ml (1.3 US pint) Rubbermaid® Servin’ Saver bowl with 25 1/8 inch holes drilled in the lid. Soil treatments included:

1. fipronil 0.1 G (low rate) 50 lb product/A (122 g/plot or 0.05 lb a.i./A)
2. fipronil 0.1 G (high rate) 100 lb product/A (244 g/plot or 0.1 lb a.i./A)
3. tefluthrin 1.5 G (low rate) 66.7 lb product/A (161 g/plot or 1.0 lb a.i./A)
4. tefluthrin 1.5 G (high rate) 133 lb product/A (322 g/plot or 2.0 lb a.i./A)
5. untreated control

Treatments were assigned to lifters according to a completely randomized design with 12 replications per treatment.
Results

Numbers and sizes of fire ant mounds around target lifter sites were recorded on 20 April, 1999, prior to any treatments being made. Based on this sample, fire ant mound density was estimated at approximately 576 mounds per acre, based on an average of 2.37 (± 0.23 SEM) mounds per plot (179 ft²).

In addition, on this date all target lifters were observed for damage to rubber boot seals on lifter armatures and to weatherproofing used on electrical outlets. Approximately 22% of the natural rubber boots used to seal the lifter armature exhibited what appeared to be fire ant chewing damage, with 1 to 3 holes per boot. On 19 September 1999, a cloudy day, fire ants were observed clustered on the accordion ridges and edges of about 8% (7 out of N=84) of the rubber boots. These ants appeared to be chewing on the rubber.

Ant activity and damage was also evident around the plugs and electrical outlets supplying power to each target lifter. Soil and debris were found in 50% of the outlets, and dead ants were found in 38.6% of outlets (N=44). Nearly 100% of the neoprene weatherproofing for electrical outlet covers was damaged by what appeared to be similar fire ant chewing behavior (Figure 3). Most significantly, in 38 out of 39 outlet boxes examined chewing damage on wires was observed (Figure 2). Of these, chewing damage in 34 (87%) boxes was sufficient to expose wiring to potential electrical shorts.

The reason for fire ant attraction to natural rubber, neoprene and wire insulation is unclear. Texture or oils found in natural rubber, might act as stimulants of fire ant chewing behavior on rubber or neoprene (S.B. Vinson, personal communication). Attraction of fire ants to electrified wires has been documented by others (e.g., MacKay et al., 1992., Slowik et al. 1997) but wiring at the Fort Wolters firing range was electrified only when the site was in use, normally only a few hours each week. There may be other, as-yet unidentified stimuli that cause fire ants to chew on rubber and plastic electrical components.

Mound counts were made on 28 March and 25 May 2000, five and eight months after treatment (MAT), 06 October 2000 (12 MAT), 10 April 2001 (18 MAT) and 09 Oct 2001 (24 MAT). Mound counts from both sample dates in the spring of 2000 were totaled for analysis, although most of the colonies observed and marked on 28 March were not active during the May sample three months later. There was a significant difference in mound counts among treatments for the Spring, 2000 sample (ANOVA, F=3.88, d.f.=4,55; P<0.0001) (Table 1); however, there were no significant difference among treatments for the 12, 18 and 24 MAT observations (P>0.05).

Significantly fewer fire ant foragers were present in vials following a two hour exposure on 01 June, 2000 (8 MAT), compared to numbers collected on 19 Oct, 1999, prior to treatment of the test area (Table 2). Lower numbers of foragers may have been due to seasonal or temperature differences, or due to treatment effects. Although there were numerically fewer fire ant foragers in treated vs. untreated plots on 01 June, 2000, the differences were not significant (ANOVA, F=0.42, d.f.=4, 55; P>0.796). There was also no statistically significant difference in foraging ant numbers among treatments in the Fall, 2000. For this reason, further foraging ant counts were not conducted after this date.
Figure 2. Neoprene weatherproofing taken from electrical outlet covers used in pop-up targets at Fort Wolters combat pistol range. Damage to foam presumed due to tunneling behavior by the red imported fire ant, *Solenopsis invicta* (image approximately life-size).

Figure 3. Arrows indicate chewing damage and exposed wiring presumed due to fire ants. Eighty-seven percent of outlet boxes had insulation chewed sufficiently to expose bare wire as in this image. Fort Wolters Army National Guard Base, Mineral Wells, Texas. 2001.
Table 1. Effect of various treatments on average numbers of active red imported fire ant mounds (± SEM) around firing range target lifters. Fort Wolters Army National Guard Base, Mineral Wells, Texas. Fall 1999 to Fall 2001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fall, 1999 Precount</th>
<th>Spring, 2000 5-8 MAT</th>
<th>Fall, 2000 12 MAT</th>
<th>Spring 2001 18 MAT</th>
<th>Fall, 2001 24 MAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>fipronil 0.05 lb a.i./A</td>
<td>2.83 ± 0.56</td>
<td>0.08 ± 0.08 b</td>
<td>0.42 ± 0.19 a</td>
<td>0.17 ± 0.11 a</td>
<td>0.25 ± 0.18 a</td>
</tr>
<tr>
<td>fipronil 0.10 lb a.i./A</td>
<td>1.92 ± 0.54</td>
<td>0.00 ± 0.00 b</td>
<td>0.08 ± 0.08 a</td>
<td>0.33 ± 0.14 a</td>
<td>0.08 ± 0.08 a</td>
</tr>
<tr>
<td>tefluthrin 1.0 lb a.i./A</td>
<td>3.00 ± 0.48</td>
<td>0.17 ± 0.11 b</td>
<td>0.00 ± 0.00 a</td>
<td>0.42 ± 0.15 a</td>
<td>0.25 ± 0.13 a</td>
</tr>
<tr>
<td>tefluthrin 2.0 lb a.i./A</td>
<td>1.92 ± 0.45</td>
<td>0.00 ± 0.00 b</td>
<td>0.17 ± 0.11 a</td>
<td>0.25 ± 0.13 a</td>
<td>0.50 ± 0.23 a</td>
</tr>
<tr>
<td>untreated control</td>
<td>2.17 ± 0.47</td>
<td>1.00 ± 0.21 a</td>
<td>0.50 ± 0.23 a</td>
<td>0.64 ± 0.20 a</td>
<td>0.25 ± 0.13 a</td>
</tr>
</tbody>
</table>

1 Numbers within the column followed by the same letter are not significantly different (P<0.0001)
2 Includes combined counts from month 5 and month 8.

Table 2. Effect of various treatments on average number of foraging red imported fire ants collected per plot in baited glass vials. Fort Wolters Army National Guard Base, Mineral Wells, Texas. March to October, 2000.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ave. no. foraging ants per plot (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19 Oct 1999 (Pre-treatment)</td>
</tr>
<tr>
<td>fipronil 0.05 lb a.i./A</td>
<td>11.60 ± 3.29</td>
</tr>
<tr>
<td>fipronil 0.10 lb a.i./A</td>
<td>14.45 ± 3.17</td>
</tr>
<tr>
<td>tefluthrin 1.0 lb a.i./A</td>
<td>20.92 ± 6.99</td>
</tr>
<tr>
<td>tefluthrin 2.0 lb a.i./A</td>
<td>10.30 ± 1.73</td>
</tr>
<tr>
<td>untreated control</td>
<td>7.18 ± 2.54</td>
</tr>
</tbody>
</table>

1 No significant differences among treatments, ANOVA, P>0.05.

No additional imported soil, dead ants or chewing damage were observed in any electrical outlets (in either treated or untreated plots) during the 18 months following replacement of outlet covers (Spring 2000 to Fall 2001). The lack of soil or chewing damage to outlets in control plots may be explained by the low fire ant populations in these plots compared to pre-treatment.

Both tefluthrin and fipronil granular insecticides may prove useful to protect electrical equipment for up to 8 months. Despite reports of longer-term efficacy for fipronil, there was no statistical evidence of its effectiveness at 12 months in this study. The small size of treated plots and slopes around the lifters may have reduced the residual effectiveness of treatments in this study.
Acknowledgments

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Literature Cited


MODIFICATIONS TO A REARING SYSTEM FOR *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE)

James T. Vogt

USDA-ARS-BCPRU
P. O. Box 67
Stoneville, Mississippi 38776

Abstract

*Pseudacteon curvatus*, dipteran parasitoids of *Solenopsis* spp., are being reared in the laboratory for release in the U.S. Large "attack boxes" serve as arenas where adult flies have access to host ants. Each attack box has a Plexiglas top through which host and parasitoid activity can be observed. The attack box contains a series of trays, each with a pair of inverted cups that alternately move up and down from 0900 to 1800 h, inducing host ants to trail back and forth as they try to remain under the cup in the down position. Difficulties that may be encountered when rearing these flies include maintaining high humidity in the attack box, condensation problems on the inner surface of the Plexiglas, and reluctance of host ants to trail when under intense fly attack. These problems were address by (1) incorporating steam-generated humidity into the attack boxes, (2) using infrared heating elements above the box to prevent condensation, and (3) restricting movement of flies in early morning until ants began trailing. Fly movement was restricted by confining newly-emerged flies in a partially-screened Plexiglas chamber, approx. 35 cm X 35 cm X 23 cm, until 0940, after ants had begun trailing back and forth within the trays. These modifications resulted in daytime RH of approx. 80-85% in the system, eliminated condensation problems, and reduced labor associated with inducing ants to trail by up to 1-1.5 person-h/d. Peak fly production in this system currently exceeds 2500 flies / box / day, sufficient for inoculative releases and biological studies.
CO-EXISTENCE PATTERNS OF ANT AND TERMITE SPECIES IN NEW ORLEANS CITY PARK

Beverly Wiltz

University of Georgia

Abstract

We described spatial and temporal patterns of ant and termite co-existence in New Orleans City Park. Studies were conducted at two spatial scales. Ants were collected monthly for one year from pitfall traps placed at 100 locations on a 5 ha section of golf course. Termites were sampled at the same 100 locations twice during the year. A park-wide species inventory is underway. Species co-existence patterns were compared for 15 sites throughout the 600 ha park.

Sixteen ant and four termite species were found at the golf course site. Brachymyrmex sp. was significantly more abundant where Solenopsis invicta was present, while Linepithema humile was less abundant. Both ant abundance and species richness were highest in May. Termites were found throughout the site and could not be correlated with ant species or abundance, in the larger scale study, we found S. invicta at 14 of 15 sites. A total of nineteen species have been identified. The site-wide abundances were positively correlated for Brachymyrmex sp. and Paratrechina sp. and for Crematogaster ashmeadi and Camponotus pennsylvanicus. A high correlation coefficient was also found for abundances of S. invicta and Pheidole sp., although the correlation was not significant at the 0.05 level.

Introduction

Competition is an important force structuring ant communities. While exotic ants have been found to reduce the abundance and species richness of native ants (Porter and Savignano 1990, Gotelli and Arnett 2000) and other arthropods (Cole et al. 1992), fire ant populations can also be impacted by competition from other insects, primarily ants (Urbani and Kannowski 1974, Apperson and Powell 1984). Many studies of species co-existence have focused on the advancing front of an invasive species, comparing invaded sites with those nearby that have not yet been invaded. Fewer studies examined long-term equilibria, if they exist. For the Argentine ant Linepithema humile and Pheidole megacephala, spatial niche partitioning can allow co-existence at a larger scale (Haskins and Haskins 1998). While either L. humile or S. invicta often appears to have displaced the other species, in some areas a dynamic equilibrium may also exist between these ants. Co-existence patterns of these species in New Orleans is of interest because the city was an early introduction site of both: the red imported fire ant was reported to be established in New Orleans by the early 1950's (Culpepper 1953) and the Argentine ant was reported in 1904 (Newell 1908).

Ants are the greatest insect enemies of termites. Despite chemical and behavioral defense mechanisms, ants could potentially play an important role in limiting the distribution of
termites through predation, competition for nesting sites, and disruption of foraging activities (Hölldobler and Wilson 1990).

The objectives of this study were to examine patterns of ant co-existence in New Orleans City Park on two spatial scales and to determine if any patterns exist in ant and termite species occurrence.

Materials and Methods

Study site: New Orleans City Park is the fifth largest urban park in the nation. It is situated on 600 ha in Mid-City, extending to just south of Lake Pontchartrain. Soils are mostly poorly drained muck soils that were former marshes. Most of the land lies at or slightly below sea level. The mean annual precipitation is 148 mm, with most occurring from April through September. Mean monthly temperatures range from 12° C in January to 27° C in August. Habitats include cypress stands, mixed hardwood forest, pond and bayou edges, golf courses, and landscaped areas.

Study 1 - Temporal and spatial patterns of ant and termite activity: This study was conducted on a 5 ha section of golf course. The site consisted primarily of turf with small stands of hardwoods and pines. Two sides were bordered by roads and a third side by a small body of water. The site was part of a larger area being monitored for subterranean termite activity. In the spring of 1999 a pine stake was driven into the ground within 2 m of each tree with diameter greater than 0.1 m. Beginning in February 2000, the site was sampled monthly for ants. Pitfall traps were placed flush with the ground, within 5 cm of the pine stake at 100 of the trees in the termite survey site. Ant traps consisted of 15 ml plastic vials containing 5 ml of ethylene glycol. An umbrella-style cover made from a 9 cm nail driven through a 4 cm x 5 cm piece of pine was placed over each to minimize debris and rainfall in the tubes. After 48 hours, vials were collected. Ants were identified using Hölldobler and Wilson (1990), Bolton (1994), and other keys.

Study 2 - Park-wide species inventory and larger scale distribution patterns: Ants are being sampled at locations representative of park habitats. Results from fifteen of the sites are presented here. Collection methods included pitfall traps, Berlese funnel, cookie crumb and cat food baits, and hand collection.

Results and Discussion

Study 1:

Sixteen ant species, belonging to five subfamilies, were collected (Figure 1). The majority of these species are insectivores or generalists. Half are exotic: Solenopsis invicta, Linepithema humile, Trichoscapa membranifera, Pseudomyrmex gracilis, Pheidole sp., Paratrechina sp., Cardiocondyla sp., and Hypoponera sp. (Smith 1979). Of the 4595 individuals collected at this site, approximately half were Solenopsis invicta. Linepithema humile and Pheidole sp. were also frequently collected but in slightly lower numbers. The number of ants collected
and species richness were highest in May (Figure 2). Species richness remained high throughout the summer, but the number of individuals declined.

The two most abundant species, the red imported fire ant *Solenopsis invicta* and the Argentine ant *Linepithema humile*, accounted for 69% of the ants collected. These invasive species have been reported to displace other ant species as well as each other (Porter et al. 1988). This did not appear to be the case at our site. While we found *L. humile* most frequently in the less disturbed area near the water, *S. invicta* was collected throughout most of the site, often in the same traps as *L. humile*. *S. invicta* was collected in 72 of the traps, *L. humile* in 53, and 28 contained both species. *S. invicta* were captured most frequently on the east side of the plot, which borders the road, while *L. humile* were found more often near the water (Figure 3). While 97% of the traps collected at least one of these species at some time, only 28% collected both. *Pheidole* sp. was consistently collected as widely as either species, and *Brachymyrmex depilis* was collected from at least 10% of the pitfall traps in all but the coldest months.

Ant abundance per trap was compared for catches with and without *S. invicta*. *Brachymyrmex* were significantly more abundant where *S. invicta* were present, while *L. humile* were significantly less abundant. No differences were found for other ant species.

Figure 1. Abundances of ant species collected in golf course study.
Subterranean termites were found infesting 29% of the trees at this site. Of the 100 trees in the survey area, *Coptotermes formosanus* Shiraki was found at 21, *Reticulitermes hageni* Banks at 5, *R. virginicus* (Banks) at 3, and *R. flavipes* (Kollar) at 1. We found no apparent spatial relationship between termite presence and ant abundance or species composition. However, every tree in the study area was within 40 m of a termite hit.

![Graph showing monthly ant abundance and species richness.](image)

**Figure 2.** Monthly ant abundance and species richness.
Study 2: Ants have been identified from fifteen of the sites. *S. invicta* were present at 14. Three species were collected that were not found in the previous study: *Camponotus* sp. 2, *Monomorium minimum*, and *Pseudomyrmex* sp. 2. The site-wide abundance were positively correlated for two pairs of species: *Brachymyrmex* sp. and *Paratrechina* sp. (ρ = 0.94949, P< 0.0001) and *Crematogaster ashmeadi* and *Camponotus pennsylvanicus* (ρ = 0.93379, P< 0.0001). A high correlation coefficient was also found for abundances of *S. invicta* and *Pheidole* sp., although the correlation was not significant at the 0.05 level (ρ=0.50481, P=0.0550).

Figure 3. Locations of pitfall traps where *S. invicta, L. humile*, both, or neither were collected. Contours indicate elevation, in meters.
References Cited


ANALYSIS OF RED IMPORTED FIRE ANT DISTRIBUTION IN ORANGE COUNTY, CALIFORNIA

Maria Diuk-Wasser, Michael Hearst and Shana Lowe

Orange County Fire Ant Authority
20541 Pascal Way, Lake Forest, CA 92630

Introduction
In October of 1998, a Las Vegas nursery discovered red imported fire ants (RIFA) in a shipment of nursery stock that originated in the Trabuco Canyon area of Orange County. The California Department of Food and Agriculture (CDFA) responded by quickly surveying the nursery, and surrounding neighborhoods. They found that a nursery, an adjacent nursery and the surrounding residential area was heavily infested. CDFA then began an extensive survey of the entire county, establishing the existence of two major infestations, one in the north county and one in the south county. In February of 2000, CDFA contracted with the Orange County Agricultural Commissioner's office, which in turn subcontracted with the Orange County Vector Control District. The Orange County Fire Ant Authority (OCFAA) was established with the mission of eradicating the red imported fire ant in Orange County. In its first year, the OCFAA had conducted over ten thousand treatments; after two years the agency had largely separated the ants from the county residents. Remaining ants are largely confined to areas that are less commonly visited.

The difficult task in eradicating the red imported fire ant, as in any eradication program, is finding and eliminating the last remaining colonies. A Geographic Information System (GIS) will allow us to focus our survey efforts in those areas most susceptible to infestation. By analyzing the current infestation and its associated habitat and incorporating information about RIFA biology, we hope to optimize our detection efforts. Among the hardest hit elements within the Southern California community is the nursery industry. The entire county is under quarantine. We hope that some of our information will allow a relaxation of the quarantine, permitting individual nurseries that are not infested, as well as areas not likely to become infested, to be excused from the quarantine requirements. In analyzing the data, we attempted to answer the following questions: How fast is the infestation spreading? Can we determine the points of introduction? How far are newly mated queens dispersing? What factors influence dispersal and successful colonization?

Materials and Methods
Our GIS consisted of OCFAA RIFA site information and a basemap that included the following layers: street information from Thomas Bros. Maps®, county land use data and vegetation maps (County of Orange Public Facilities and Resources Department, Geomatics and Land Information Systems), and county parcel data from Digital Map Products. We also incorporated CDFA survey data (from CDFA countywide surveys), grading site information derived from Orange County grading permits, and U.S. Geological Survey (USGS) pitfall trap data. USGS has been conducting ant surveys throughout Orange County since the winter of 1999. The project monitors 159 trap arrays, each with five pitfall traps. Each site is checked for both winter and summer species. USGS and the OCFAA have worked closely in the sharing of information.
OCFAA follows a work plan written by CDFA, based on recommendations provided by a science advisory panel consisting of experienced fire ant researchers. When a site is determined positive for RIFA, it is treated every three months for a year. Three months after the last treatment, the site is surveyed. If ants are found at any point, the site undergoes a second treatment cycle; if no ants are found, the site is surveyed every six months for a period of two years. RIFA data was collected by OCFAA technicians, using Trimble® GeoExplorer Data Collection Systems and downloaded into GPS Pathfinder Office®. This software provides the functionality to correct, view, and edit GPS data collected in the field. The data is imported into ESRI ArcView® 3.2 GIS software for query, mapping, and analysis, as needed. For the data analysis, we used ArcView® extensions (Spatial Analyst and USGS Animal Movement) and scripts written for ArcView (Point Proximity Analysis (Nick Bauer, 2001), Count Points in Polygons (Yingming Zhou, 2000) and Clip Grid (Tom Van Niel, 2000)). It should be noted that the 65,000 site records used in this analysis were originally gathered as part of our field operations. This information was not gathered for research purposes, but contained a vast amount of useful data.

Distribution and infestation patterns
To examine RIFA spread, we calculated the number of new sites found every 60 days, beginning in August 2000, and analyzed the infestation level throughout Orange County. To describe the distribution patterns, we plotted the known sites in August 2000, April 2001, and April 2002 over the state grid system. We then calculated the number of sites per each square mile cell, using the “Count Points in Polygons” script.

Introduction points
We examined the density of points in the areas where RIFA introductions were suspected, based on anecdotal information. If these were indeed the introduction points, we expected them to be among the areas of highest density, with density decreasing as the distance from the suspected introduction area increases. To obtain the point density, we used ArcView Vector Conversion Process, which converts the point feature layer to ArcView Grid format. We used the state grid system to define cell location and size of the output density grid.

Dispersal of newly-mated queens
To explore dispersal distance of newly mated queens, we did a proximity analysis to determine how far each RIFA site is to its closest neighbor. We used the “Point Proximity Analysis” script, which creates buffers around each RIFA site at a specified distance. Each buffer is then analyzed to see if another site exists within the buffer. Previous research (Vogt et al 2000) showed that newly mated RIFA queens are probably limited to flight distances of no more than 5km. Due to the great differences in relative humidity between Southern California and the southeast U.S., we believed that the flight distances would likely be far less. We chose a buffer distance of 1km. If this previous research is correct, the great majority of our sites should fall within these buffer zones.

Correlates of RIFA presence/abundance
We created an overall risk assessment map, using ArcView’s Weighted Overlay Process within ArcView’s Spatial Analyst Extension. The Weighted Overlay Process combines values in multiple input grid themes and creates an output theme. To combine themes with different types of data, cell values are converted to common units. The new units assigned are based on a numeric evaluation
scale (ex.1-9, 1-5, etc). Each theme is then given a weight, based on its estimated level of influence. These weighted values are then added together to create one output theme.

The factors used in this analysis were habitat type (derived from county vegetation maps), habitat disturbance (derived from grading permits), and proximity to nearest RIFA site (based on our analysis). These factors were believed to influence RIFA dispersal, based on a comparison of these independent factors with RIFA site density. We first converted all layers to grid format. Habitat type was converted to a grid theme, using the “Convert to Grid” application in Spatial Analyst. We then created a disturbed habitat grid theme by plotting the number of grading sites per grid. We also created a proximity grid by making a 1km buffer around each site, then converting the buffers to a grid theme and assigning two values, one for cells within the buffer and another for cells outside the buffer.

**Results and Discussion**

*Distribution and infestation patterns*

USGS pitfall trap data in natural areas indicated that RIFA is confined to disturbed and irrigated habitat. Orange County is surrounded by dry, natural areas to the east and south, which would therefore restrict its dispersal in those directions.

Between February 2000 and August 2000, we visited the original CDFA sites. All new sites after that date are either new colonies or colonies that had not been detected in previous surveys. It is impossible to distinguish between these two cases. However, survey efforts have steadily increased since the inception of the program, through an increase in staff and more efficient and productive survey methods. If the fire ant population had remained the same or increased, we would expect to find a significant number of new sites. In contrast, the number of new sites has decreased over time (Figure 1). The decrease in new sites during the winter months may be explained by seasonal fluctuations in ant activity and by a decrease in OCFFA survey staff. The difference in the new site discoveries between the winter of 2000 and the winter of 2001 may also be explained by a significantly greater amount of rainfall in the winter of 2000, reducing survey efforts. As the program progresses and more data is compiled, these patterns will become clearer.

![Figure 1. Number of new RIFA sites discovered](image)

60
Figure 2 shows that the distribution of fire ant sites throughout Orange County is concentrated in two main clusters. Most of the new sites have been discovered within these two general areas, in spite of heavy surveys throughout the entire county. The sites represented in these maps are in different stages of the treatment protocol. Currently, only 3.62% of the sites that have completed the treatment cycle are still positive. The sites showing no apparent RIFA activity will be surveyed every six months for two years; at which point, they will be considered free of fire ants. Therefore, these maps should not be interpreted as representing current distribution, as many of these sites are no longer active.

Figure 2. Density of RIFA sites in (a) August 2000; (b) April 2001; (c) April 2002

Introduction points
During its yearlong survey of the entire county, CDFA determined that the most mature infestations were found at a golf course and an adjacent racetrack in the north county, as well as two contiguous nurseries in the south county. It has been assumed that these were the original points of introduction. We combined these suspected points of introduction with a map showing RIFA site density (see Figure 3). The density pattern observed is consistent with what would be expected of a natural dispersal from these points.
Figure 3. Suspected points of fire ant introductions and fire ant density in April 2002

*Dispersal of newly-mated queens*

The proximity analysis showed that 95.6% of sites are within 1 km of another site. This may indicate that naturally dispersing queens are not traveling long distances. Direct measurement of flight distances should be conducted to confirm this suspicion. If it is shown that newly mated queens in Southern California are dispersing less than 1 km, it may be possible to modify the existing nursery quarantine.

*Correlates of RIFA presence/abundance*

Figure 4 represents the overall risk of fire ant infestation in Orange County, derived from the Weighted Overlay. Habitat type was given the highest weight, followed by proximity, and then habitat disturbance. The darker grids represent the areas at highest risk of infestation. This information will be useful in planning survey strategies. As we come to the point in our program when remaining colonies are in more remote locations, it will become helpful to prioritize our survey efforts based on this overall risk assessment.
Conclusions
Utilizing a GIS has allowed for better dissemination of information. We have provided maps, illustrating the infestation status to elected officials and have been able to report our progress to CDFA. We are also able to better plan our treatment and survey activities. We are hopeful that further analysis of the available data will provide a predictive component, as well. We view the work presented here as a preliminary study for future analysis. We plan to do more intensive spatial analysis of the data and develop a model within the GIS to help us achieve our ultimate goal of eradication.

Acknowledgements
We would like to thank the following people for their support: the technical staff at the Orange County Fire Ant Authority for the collection of data, OCFAA Program Manager Richard Bowen, OCVCD District Manager Dr. Robert Sjogren, OCFAA Technical Advisor Dr. Richard Meyer, OCFAA Office Manager Michelle Harrison, Dr. Les Greenberg of the University of California at Riverside, and the staff of the California Department of Food and Agriculture.

References Cited
TEACHING MATERIALS FOR IMPORTED FIRE ANT TRAINING PROGRAMS

K. L. Flanders¹, L. C. (Fudd) Graham¹, V. E. Bertagnolli¹, R. N. Ward², K. E. Ward² and K. W. Creel³

¹Department of Entomology and Plant Pathology, Auburn University
²Alabama A&M University
³Alabama Cooperative Extension System

The goal of this project is to provide training on sustainable, site-specific management of imported fire ants. Grants from the Southern Region SARE Professional Development Program and the Alabama Fire Ant Management Program were used to develop training materials. County Extension Agents helped design and develop the materials, which include PowerPoint presentations, videos, posters, mound models, and specimen mounts. These supplement four Alabama Cooperative Extension System publications and two regional publications on fire ants. County agents are now using these materials to teach other trainers and influential stakeholders about sustainable fire ant management. For more details, see our web site at www.aces.edu/dept/fireants.

The PowerPoint presentations are useful for civic clubs and similar organizations. They are also appropriate for middle school- and high school-age students. There are extensive notes that provide background information for use in making the presentations. Presentations are available as slides or on a CD. An additional CD will have supplementary materials on fire ant identification and biology.

There are four posters (42" X 36"), each covering a different topic: fire ant baits, biological control of fire ants, colony establishment, and life in the fire ant mound. These posters are useful for civic clubs and similar organizations. They are also appropriate for middle school-and high school-age students. They work well as exhibits because they are self-explanatory. Fire ant circles, embedded in clear acrylic, contain examples of the different sizes of adult fire ants.

The fire ant videotape is useful for civic clubs and similar organizations. It is also appropriate for middle school-and high school-age students. They videotape includes three video presentations on fire ant management and biology. One of the management videos has been modified for use in the Texas Fire Ant Management Program. The video presentation on fire ant biology that is included on the tape was produced by the University of Arkansas Cooperative Extension Service.

Six publications on fire ants are available, in cooperation with the University of Arkansas Cooperative Extension Service and Texas Agricultural Extension Service. Felt models (27" X 36") are designed for use in presentations with young children. Velcro-backed plastic ants and labels can be moved around the model. Three-dimensional models are designed with peel-away "soil" so children can see inside the fire ant mound where there are interconnecting tunnels and chambers.
A QUEEN PHEROMONE INDUCES WORKERS TO KILL SEXUAL LARVAE IN COLONIES OF THE RED IMPORTED FIRE ANT (SOLENOPSIS INVICTA)

Richard J. Deslippe1, Emily A. Klobuchar1, Daniel Velasquez2 and Robert Renthal2

1 Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131
2 Biology, University at Texas at San Antonio, 6900 N. Loop 1604W, San Antonio, TX 78249

Abstract: We conducted a series of bioassay to study how queens control worker execution of sexual larvae in colonies of the red imported fire ant, Solenopsis invicta. In all experiments, subset colonies were made from many larger polygyne colonies, and each unit included 20 sexual larvae that were monitored over the course of 48 to 96 h. The larvae mostly survived in queenless colonies, but were mostly killed in queenright colonies, regardless of whether or not the queen was fertilized. The larvae were also killed when corpses of queens were added to queenless colonies. Whereas queen extracts in acetone did not induce killings significantly, such extracts in buffered saline led workers to execute most sexual larvae, indicating successful extraction of an execution pheromone. We identified the probable storage location of the chemical as the poison sac, and found both fresh (1 d) and old (21 d) extract kept at room temperature to be equally effective in inducing executions. Extracts of both winged queens (which do not induce killings) and wingless queens were analyzed by polyacrylamide gel electrophoresis. The latter contained two bands not present in the former: a 14 kDa protein and a 1-3 kDa peptide. Either or both of these bands may be related to the execution pheromone. The 14 kDa protein is in the size range of insect pheromone-binding proteins and lipophilic molecule-binding proteins found in secretory glands of other organisms. The 1-3 kDa band is in the size range of peptide pheromones known in various organisms, including insects.
THE ECONOMIC ASSESSMENT OF RED IMPORTED FIRE ANTS IN TEXAS

Kerinne Schroeder, Curtis Lard, Victoria Salin, and Sara Robison

Texas A&M University, Dept. of Agricultural Economics, College Station, TX 77843-2124

David Willis

Texas Tech University, Dept. of Applied and Agricultural Economics, Lubbock, TX 79409-2121

Abstract

The red imported fire ant (Solenopsis invicta) has become a major economic pest in various sectors of the Texas economy. In order to estimate the economic impact of this pest throughout the state on selected sectors, two studies were conducted: an urban study and an agriculture study. The primary data used in the statewide economic analysis were gathered through five different sector surveys. Single-family households, golf courses, schools, and cities sector data were collected for the urban study for 1998. The fifth survey, the agriculture sector, was conducted in 2000 to obtain fire ant expenses for the calendar year of 1999. The economic impacts of fire ants for the sectors surveyed were expanded to include all affected areas of Texas, and the expenditures for other sectors not surveyed were extrapolated from the data collected in the urban and agriculture studies.

These studies estimated that fire ant damages and expenditures exceed $1.2 billion in Texas annually. The household sectors (single-family and multi-family residences) had the greatest expenditures for fire ant control and treatment with 59 percent ($711.5 million) of all expenses due to fire ants in Texas. Another study conducted by Texas Tech University found that the electrical and communication sector expenses were estimated to be $146.5 million annually (Teal et al., 1999). The agriculture sector had an estimated $90.57 million (7.5 percent of statewide fire ant expenditures) in expenses due to fire ants in Texas. The expenditures due to fire ant damages and control measures for the remaining sectors such as commercial businesses, sod producers, nurseries, multi-family households, institutions, churches, cemeteries, and airports were estimated to be more than $256 million.

Fire ant expenditures were estimated for two broad categories; treatment expenses and other item expenses. Treatment expenses included the costs for insecticide mound treatment, insecticide baits, and other “organic” treatments. Other item expenses included supplies, like gloves or repellent sprays; equipment, like sprayers or spreaders, labor; and any professional services. Treatment expenditures accounted for 49 percent ($518.9 million) of all expenses due to fire ants (excluding the electrical and communication sector). The multi-family household, city, airport, commercial business, church, and cemetery sectors spent an estimated 65 percent of total fire ant expenses on treatment. Golf courses spent only 10.8 percent on treatments, because they had huge repair costs due to fire ant damages to irrigation systems. The agriculture sector had an estimated $74.55 million (82 percent) in other item expenses and $16 million (18 percent) due to treatment expenses. The agriculture study was a cooperative effort that involved Texas Tech University, Texas A&M University, and Texas Agricultural Statistical Service (TASS).
Introduction

Red Imported Fire Ant \( [\text{Solenopsis invicta} \text{ Buren (Hymenoptera: Formicidae)}] \) infestations were first documented in Texas in 1953, and today over 56 million acres are infested with fire ants. To control the spread of fire ants, the Texas Department of Agriculture initiated a county quarantine program in 1958 when it quarantined the state’s six most eastern counties. However, the fire ant quarantine failed to stop the westward spread and today 160 Texas counties are under quarantine (Texas Imported Fire Ant Research & Management Plan, 1996).

In 1997, in order to both control the spread and to document the severity of the fire ant infestation, the Texas legislature funded a 6-year red imported fire ant initiative at an annual rate of $2.5 million. The specific objectives of the legislative initiative were to document the economic and biological severity of the infestations and to discover appropriate and cost-effective management programs that may alleviate future damage from this pest. This on-going program has been a multi-disciplinary effort, involving both the rural and urban sectors of the state. This paper presents results from the economic component of the initiative’s first and second two-year phases (Salin et al., 2000; Willis et al., 2001).

The specific objectives of the economic component of the Texas Fire Ant Project include:

1. To identify items that are considered expenditures or costs, and those items that have value or benefits, which are associated with the control and management of fire ants.
2. To estimate the cost of various control and management measures used by homeowners, golf courses, schools, cities, agricultural producers, and other major sectors in Texas.
3. To discover the various ways in which, and areas where, the fire ant affects the urban and agricultural space.
4. To estimate various expenditures associated with fire ant damages.
5. To estimate the overall economic impacts associated with fire ants in metroplexes, rural areas, and agriculture.
6. To serve as a benchmark to evaluate possible control and management programs implemented by the Texas Fire Ant Project.

Materials and Methods

The primary data used in the economic analysis were gathered through five different sector surveys. Single-family households, golf courses, schools, and cities sector data were collected during the urban study for 1998. The fifth survey, the agriculture sector, was conducted in 2000 to obtain fire ant expenses for the calendar year of 1999. Table 1 summarizes the survey design and procedures for the five sectors surveyed.
Table 1.1. Summary of Survey Design and Procedures for the Economic Impact Study

<table>
<thead>
<tr>
<th></th>
<th>Households</th>
<th>Golf Courses</th>
<th>Schools</th>
<th>Cities</th>
<th>Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size (n)</td>
<td>272</td>
<td>48</td>
<td>52</td>
<td>5</td>
<td>3612</td>
</tr>
<tr>
<td>Sampling Technique</td>
<td>Area frame</td>
<td>Randomized quota sampling</td>
<td>Randomized quota sampling</td>
<td>Census</td>
<td>Area frame</td>
</tr>
<tr>
<td>Source of Expansion Factors</td>
<td>TASS</td>
<td>Texas Golf Course Directory</td>
<td>Texas Education Agency</td>
<td>Does Not Apply</td>
<td>TASS</td>
</tr>
<tr>
<td>Survey Administration</td>
<td>TASS telephone</td>
<td>TAMU mail/telephone</td>
<td>TAMU mail/telephone</td>
<td>TAMU mail/telephone</td>
<td>TASS personal interview</td>
</tr>
</tbody>
</table>

Urban Study. The data collection phase of the urban study for the principal metropoles in Texas was completed in 1998 and 1999, and included costs and benefits associated with the calendar year 1998. The costs, practices, and benefits were collected for single family homes, schools (public and private schools, including elementary schools, high schools, and higher education institutions), cities, and golf courses (private and public). The primary data were collected from households, schools, cities, and golf courses by using carefully structured questionnaires. The main goal of the survey for each sector was to obtain a representative sample of the households, schools, and golf courses in Texas.

Trained personnel from Texas Agricultural Statistical Service (TASS) conducted the sampling and personal interviews to obtain household survey responses. The single-family households were stratified by metroplex using the TASS geographic area frames. The area frames were modified with the 1997 Census of Agriculture, which indicated non-agricultural areas, primarily residences. These adjustments were necessary because the area frame sample used by TASS was designed to obtain data about acreage, yields, numbers, etc. of agricultural products and inputs.

Questionnaires were administered by mail to schools, cities, and golf courses with phone follow-ups. All five metro cities (Austin, Dallas, Fort Worth, Houston, and San Antonio) were surveyed. Questionnaires were sent to the city manager’s office or the appropriate city department responsible for utilities, parks, and city grounds in the five metro cities. Sample size was determined by using the usual scientific statistical procedure as follows:

\[ n = \frac{z^2 \sigma^2}{e^2} \]

when \( n \) = sample size
\( z \) = number of standard deviations from the population mean
\( \sigma \) = the population standard deviation, and
\( e \) = the accepted error or desired level of precision.
It was decided that an "e" of plus or minus 10 percent for the estimated value (sample mean) for fire ant expenditures was acceptable. Thus, a sample size sufficient to assume that the estimated sample mean was within 10 percent of the population mean was specified. The z value also was based upon a 10 percent level of precision. An estimate for \( \sigma \) was approximated by the use of range tests. This technique also guided the determination of survey size for golf courses and schools.

The types of data collected for each of the four sectors in the urban study included: (1) characteristics of each entity, (2) defining the fire ant problem, (3) identifying types of expenditures for control and management, (4) maintenance expenditures and investments, (5) medical expenditures, (6) damages to electrical type equipment, and (7) general information on the sector. The complete text of the questionnaires is available from the authors upon request.

**Agriculture Study.** The Department of Agricultural and Applied Economics at Texas Tech University and the Department of Agricultural Economics at Texas A&M University joined together to conduct the agriculture study. The agriculture survey reports costs and benefits for the 1999 calendar year. A structured questionnaire was developed in association with trained personnel from TASS and Texas Tech University. The primary objective of the sample design was to obtain a representative sample of Texas agricultural producers. To achieve this goal, a sampling scheme based upon the TASS's sampling procedure was utilized. The TASS area frame sampling procedure provides accurate regional estimates on acreage, yields, numbers of producers, and input use. TASS administered the required personal interviews as an addendum to their 1999 Fall Area Survey. Over 4,000 completed surveys were received from TASS and 3,612 usable responses were analyzed in the agriculture statewide estimate.

Secondary data were used to augment the survey data. Secondary data sources used consisted of the 1997 United States Census of Agriculture and other data annually compiled by TASS. TASS also provided the expansion factors (weights) for aggregating the survey data into damage estimates for each of the fifteen Texas agricultural statistics districts, in addition to a statewide total.

The types of data collected from each agricultural producer included: (1) irrigated and non-irrigated acres of cropland, (2) crop losses related to fire ants, (3) livestock losses related to fire ants, (4) equipment repair costs due to fire ants, (5) equipment replacement cost due to fire ants, (6) fire ant damages to the farmstead, (7) fire ant related medical expenditures, (8) fire ant related veterinary expenditures, (9) cost of fire ant control materials, (10) special equipment purchased to apply fire ant control materials, and (11) the agricultural benefits (if any) of fire ant infestations. The complete text of the questionnaire is available from the authors upon request.

**Aggregation Methods.** The other sectors' fire ant expenditures in the statewide economic impact analysis were extrapolated from the data collected in the five surveys. The expense of creating and conducting new surveys to obtain data for each of the other nine sectors would be very costly. Therefore, the data from the five prior surveys were used to extrapolate and aggregate

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1 Other sectors include multi-family households, nurseries, sod producers, commercial businesses, institutions, airports, churches, and cemeteries. The electric/communication sector expenses were estimated according to the Texas Tech University report (Teal et al, 1999).
expenditures and damages to all other sectors to obtain a statewide estimate of fire ant expenditures.

The study collected only the original data, which were used along with secondary data, for sectors that were not surveyed. The final statewide estimate is an annual estimate, but is derived from original and secondary data that were not all from the same year. Because fire ants are biological creatures, their effects and associated spending by consumers, businesses, and public sector authorities almost certainly vary according to the weather. Therefore, it would not be valid to say this estimate is for any one year. However, this is the best benchmark available.

**Results and Discussion**

Fire ants in Texas have a tremendous economic impact on the various sectors of the economy. This scientific study has identified and quantified many of these impacts. The impacts are given by geographic area and sector, as well as urban and rural areas.

**Geographic Areas.** Fire ant damage in Texas is not uniformly spatially distributed over the entire state. The statewide economic impact of fire ants was estimated by expanding the information obtained from the surveys across the affected counties in Texas. These surveys included urban sectors and rural sectors. Figure 1 shows the Texas Red Imported Fire Ant Quarantine Area and the seven additional counties that reported damages in the agriculture survey are the counties that were considered to be affected for this study. Counties indicated in black are quarantined for fire ants and counties in gray stripe reported fire ant damages in the agricultural survey. For this study it was assumed that the counties that were not part of the affected area had no economic impact from fire ants and no damages were calculated for these 87 counties.

![Figure 1. Texas Fire Ant Quarantine Area and Counties with Reported Fire Ant Damages from the Agriculture Survey](image)

**Statewide Economic Impact.** The total damages and expenditures from red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Texas were estimated to be $1.2 billion.
annually (Table 2). Residential households suffered the greatest expense with over 50 percent of the total statewide annual costs. The survey results summarized in this report probably underestimate the statewide costs, because not all costs were considered for several other sectors that could have significant costs due to fire ant damage and control. Sectors not taken into consideration included game and wildlife, highways, roadsides, racetracks, resorts, and theme parks. Table 2 includes the various damages and expenditures by sector of the economy.

Table 2. Annual State Total Fire Ant Damages & Expenditures by Sector for Texas

<table>
<thead>
<tr>
<th>Sector</th>
<th>Damages &amp; Expenditures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Agriculture</td>
<td>$90,572,032</td>
</tr>
<tr>
<td>2. Airports</td>
<td>26,620,789</td>
</tr>
<tr>
<td>3. Cemeteries</td>
<td>63,922,406</td>
</tr>
<tr>
<td>4. Churches</td>
<td>9,455,328</td>
</tr>
<tr>
<td>5. Cities</td>
<td>1,127,469</td>
</tr>
<tr>
<td>6. Commercial Businesses</td>
<td>45,898,370</td>
</tr>
<tr>
<td>7. Golf Courses</td>
<td>47,294,894</td>
</tr>
<tr>
<td>8. Institutions</td>
<td>130,793</td>
</tr>
<tr>
<td>9. Multi-family Households</td>
<td>9,178,695</td>
</tr>
<tr>
<td>10. Nurseries</td>
<td>5,524,861</td>
</tr>
<tr>
<td>11. Residential Households</td>
<td>702,356,668</td>
</tr>
<tr>
<td>12. Schools</td>
<td>42,253,421</td>
</tr>
<tr>
<td>13. Sod Producers</td>
<td>13,371,468</td>
</tr>
<tr>
<td>14. Electric and Communications*</td>
<td>146,500,000</td>
</tr>
<tr>
<td>Statewide Total</td>
<td>$1,204,207,194</td>
</tr>
</tbody>
</table>

* Estimate taken from Teal et al., 1999.

Single-Family Household Sector. Fire ant expenditures and damages were reported for MSAs (Metropolitan Statistical Area), intermediate MSA counties, and rural counties that were in the affected areas. The single-family household sector included both single attached and single detached household units. The greatest expense of all the sectors analyzed was the single-family residential sector with an estimated $702.4 million annual in expenditures due to fire ants. Expenditures were estimated as follows: treatment expenses, such as insecticide and baits, $364.1 million; and other expenses, including heating and air conditioning equipment repaired or replaced, $338.2 million. The weighted average cost of fire ant expenditures for single-family households annually was $151. Table 3 outlines the various expenditures by geographic area for single-family households.
Table 3. Single-Family Household Expenditures due to Fire Ants by Geographic Area and Type of Expense

<table>
<thead>
<tr>
<th>Area</th>
<th>Treatment Costs</th>
<th>Other Costs</th>
<th>Total Expense</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Antonio</td>
<td>$66,053,861</td>
<td>$131,578,597</td>
<td>$197,632,458</td>
</tr>
<tr>
<td>Dallas</td>
<td>$90,243,576</td>
<td>$9,102,782</td>
<td>$99,346,358</td>
</tr>
<tr>
<td>Houston</td>
<td>$65,112,365</td>
<td>$45,943,031</td>
<td>$111,055,396</td>
</tr>
<tr>
<td>Fort Worth</td>
<td>$36,615,322</td>
<td>$35,450,001</td>
<td>$72,065,323</td>
</tr>
<tr>
<td>Austin</td>
<td>$21,608,348</td>
<td>$24,174,772</td>
<td>$45,783,120</td>
</tr>
<tr>
<td>Intermediate MSA</td>
<td>$66,783,279</td>
<td>$81,543,462</td>
<td>$148,326,741</td>
</tr>
<tr>
<td>Rural Counties</td>
<td>$17,725,881</td>
<td>$10,421,391</td>
<td>$28,147,272</td>
</tr>
<tr>
<td>Total Expenditures</td>
<td>$364,142,632</td>
<td>$338,214,036</td>
<td>$702,356,668</td>
</tr>
</tbody>
</table>

Golf Course Sector. Fire ant expenditures and damages for golf courses were reported for MSAs and for the non-MSA counties included in the affected areas. The study involved all golf courses in Texas, including private, public, municipal, and all other types of golf courses. The estimated number of golf courses located in the fire ant affected area of Texas was 706 for this study. Repairs to equipment and courses, replacements of damaged equipment and irrigation systems, treatment measures, and medical costs for golf course owners were an estimated $47.29 million annually.

Replacement and repair costs were often expensive, accounting for 89 percent of the $47.29 million worth of expenditures caused by fire ants to Texas golf courses. Replacement costs were the single greatest expense for the golf course sector with an estimated $40.14 million, with a majority of the cost associated with the replacement of irrigation systems. Repair expenses were estimated to be $2.02 million, and treatment expenses totaled an estimated $5.13 million, while medical expenses were estimated to be only $6,205. The weighted average cost of fire ant expenditures for golf courses annually was $66,990. Table 4 outlines the various expenditures by geographic area for the golf course sector.

Table 4. Golf Courses Expenditures due to Fire Ants by Geographic Area and Type of Expense

<table>
<thead>
<tr>
<th>Area</th>
<th>Repair</th>
<th>Treatment</th>
<th>Replacement</th>
<th>Medical</th>
<th>Total Expenses</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Antonio</td>
<td>$316,123</td>
<td>$260,660</td>
<td>$900</td>
<td>$309</td>
<td>$577,992</td>
</tr>
<tr>
<td>Dallas</td>
<td>$228,307</td>
<td>$571,375</td>
<td>$9,210,163</td>
<td>$0</td>
<td>$10,009,845</td>
</tr>
<tr>
<td>Houston</td>
<td>$333,167</td>
<td>$1,619,971</td>
<td>$1,583,623</td>
<td>$849</td>
<td>$3,337,610</td>
</tr>
<tr>
<td>Fort Worth</td>
<td>$187,899</td>
<td>$495,292</td>
<td>$13,501,078</td>
<td>$890</td>
<td>$14,185,159</td>
</tr>
<tr>
<td>Austin</td>
<td>$868,962</td>
<td>$2,031,018</td>
<td>$14,904,658</td>
<td>$2,594</td>
<td>$17,807,232</td>
</tr>
<tr>
<td>Non-MSA Counties</td>
<td>$2,019,059</td>
<td>$5,126,697</td>
<td>$40,142,933</td>
<td>$6,205</td>
<td>$47,294,894</td>
</tr>
</tbody>
</table>

Agriculture Sector Study. The economic impact of fire ant damages exceeded $90 million for Texas agricultural producers in 1999. Fire ant damages are reported for nine damage categories as follows: crop yield losses were $33.4 million, control costs were $16.02 million, equipment repair costs were $17 million, equipment costs were $1.66 million, farmstead damages were $9.1 million, equipment replacement costs were $7.4 million, livestock losses were $4.6 million,
medical expenses were $0.56 million, and veterinary costs were $0.86 million. There is an ongoing debate concerning the existence of potential agricultural benefits from fire ant infestations. Some researchers hypothesize that fire ants prey on agricultural pests such as boll weevils and corn earworms. If this benefit can be documented, it could offset some of the damages associated with fire ants in some areas of the state. A $1.54 million statewide benefit was derived from the ten respondents who were able to quantify the benefit value. Less than 10% of the respondents who stated that there were beneficial effects were able to provide a dollar value. If this benefit is considered to be similar for the producers who indicated unquantifiable beneficial effects, then the overall benefit would be over $15 million annually. Table 5 outlines the various expenditures by type of loss and expenditures for the agriculture sector. Figure 2 illustrates the expenditures due to fire ants for the agriculture sector by Texas Agricultural Statistics District.

Table 5. Texas Fire Ant Damages for 1999 by Loss and Expenditure Category for Agriculture

<table>
<thead>
<tr>
<th>Damage Category</th>
<th>Damage</th>
<th>Percentage of Total Damage</th>
<th>Number of Farms Reporting Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop Yield Loss</td>
<td>$33,441,777</td>
<td>36.92%</td>
<td>259</td>
</tr>
<tr>
<td>Livestock Loss</td>
<td>$4,627,030</td>
<td>5.11%</td>
<td>211</td>
</tr>
<tr>
<td>Repair Cost</td>
<td>$16,956,961</td>
<td>18.72%</td>
<td>375</td>
</tr>
<tr>
<td>Replacement Cost</td>
<td>$7,390,836</td>
<td>8.16%</td>
<td>135</td>
</tr>
<tr>
<td>Farmstead Cost</td>
<td>$9,056,496</td>
<td>10.00%</td>
<td>240</td>
</tr>
<tr>
<td>Medical Cost</td>
<td>$561,356</td>
<td>0.62%</td>
<td>87</td>
</tr>
<tr>
<td>Veterinary Cost</td>
<td>$860,856</td>
<td>0.95%</td>
<td>40</td>
</tr>
<tr>
<td>Control Cost</td>
<td>$16,019,737</td>
<td>17.69%</td>
<td>1,181</td>
</tr>
<tr>
<td>Equipment Cost</td>
<td>$1,656,983</td>
<td>1.83%</td>
<td>113</td>
</tr>
<tr>
<td>State Total</td>
<td>$90,572,032</td>
<td>100.00%</td>
<td>1,358</td>
</tr>
</tbody>
</table>

Note: The farm survey consisted of 3,612 observations.

1The value for the total number of farms reporting damages is not equal to the sum of the number of observations in each damage category. The State total value is the number of surveyed farms reporting at least one type of damage, but many farms reported more than one type of damage.
Other Sectors. Other sectors included in the economic impact of red imported fire ants are airports, cemeteries, churches, commercial businesses, institutions, nurseries, and sod producers. The greatest estimated expenditure of $63.9 million was for cemeteries, followed by commercial businesses with an estimated cost of $45.9 million. Airports had estimated expenses of $26.6 million, and expenses for churches were estimated to be $9.5 million. Texas nurseries and sod producers were estimated to have annual expenditures to control and manage fire ants of $5.5 million and $13.4 million, respectively.

Electrical and Utilities Study. A corollary study conducted by Texas Tech University (Teal et al.) examined costs associated with fire ant damages to electrical and communications equipment. That report found that fire ant related damages sustained within Texas to electrical and communication equipment totaled $146.5 million per year. The statewide total expenses reported here include electrical and communications expenses.

Conclusions

The survey findings reported in this paper indicate the perceived economic significance of the red imported fire ant as a pest in Texans. Furthermore, as the fire ant continues to move farther north and west, these damages can be expected to increase if there is no coordinated action to control them. Annual expenditures were estimated to be in excess of $1.2 billion for fire ant damages and control costs in Texas. The initial assessment of economic impact serves as a baseline for evaluation of the benefits of new control and management programs, such as the community-based effort underway as part of the Texas Imported Fire Ant Research and
Management Plan. The work demonstrates the importance of sound economic input into interdisciplinary research and extension programs.

These surveys provide the only comprehensive effort to collect primary data on the economic effects of fire ants. The survey methods and aggregation procedures provide a strong scientific basis for investigation of the pest problems and the value of improved management programs.

In addition to the quantitative estimates of costs and control expenditures, both surveys highlight the continuing need for understanding non-monetary implications of the fire ant. It is a research challenge to understand the significance of problems that are not easily put into monetary figures. For example, respondents to these surveys provided input into the non-monetary benefits of effective control of the fire ant. Willingness to pay for effective control averaged $89, substantially below actual household expenditures. The contingent valuation results are puzzling and the conclusions should not be overstated given the stated difficulty of several respondents in answering these questions.

A close working relationship between economists and scientists researching new control technologies and management practices documented the costs, and provided valuable insight into the costs effectiveness and benefits of collaborative programs. This report will serve as a benchmark for future research, measuring the effectiveness of any organized community, county, or statewide control and management programs. The information provided by this study will be valuable to many others, such as entomologists, economists, households, chemical companies, policymakers, and government officials of Texas. This study and its results may also be valuable to other researchers and areas of the United States where fire ant infestations occur.
References Cited


“Texas Imported Fire Ant Research & Management: Together We Can Lessen the Sting of the Fire Ant Problem.” 1996. A joint effort by Texas Agricultural Experiment Station, Texas Agricultural Extension Service, Texas Department of Agriculture, Texas Parks and Wildlife Department, Texas Tech University, and The University of Texas.


Willis, D., V. Salin, and C. Lard. 2001. “Economic Impact of Fire Ants to Texas Agriculture” Selected paper at meeting of the Southern Agricultural Economics Association, Fort Worth, TX.
Abstract. The parasitic phorid fly, *Pseudacteon tricuspis* was found to be attracted to the volatile compounds released by shaken fire ant workers. Among the compounds released were alarm pheromones, venom alkaloids, and recruitment pheromones. In subsequent bioassays we eliminated poison sac contents and recruitment pheromones as possible attractants. We have demonstrated the involvement of alarm pheromones in the attraction of this phorid fly to their host fire ant.

Introduction. The imported fire ants, *Solenopsis invicta* and *S. richteri*, currently inhabit over 125 million hectares in Puerto Rico and twelve southern states from Texas to Virginia (Lofgren 1988). *Solenopsis invicta* has also become established in limited areas in California, Arizona, and New Mexico. Annual damage, treatment, and medical costs are reported to exceed a billion dollars. Fire ant populations in the U.S. are 5-7 times higher than in their native range of Brazil and Argentina, likely the result of escape from natural enemies left behind in South America (Porter et al. 1992).

At least 18 species of *Pseudacteon* flies have been found attacking fire ants in South America (Porter and Pesquero 2001). The flies are highly specific in their host preferences and they have been shown to stop fire ant foraging and may shift the local competitive balance to other ant species (Porter et al. 1995).

Maggots of these miniature flies develop in the heads of fire ant workers, decapitating their host upon pupation. The maggot then pushes the mouthparts aside and pupates within the empty head capsule using it as a pupal case. The worker ants respond to this "dead ant" by taking it out of the colony onto a refuse pile. Here the adult fly ecloses and starts the cycle all over again (Porter 1998).

The flies are able to locate their fire ant hosts. Fire ant pheromones (kairomones in the context of parasite host finding) are probably involved in phorid fly flight activation, attraction, and initiation of attack. Elucidation of the fly/host semiochemical interactions could lead to better utilization of this suite of fire ant parasites.

Previous investigations demonstrated that electrically stimulated fire ant workers release all detectable semiochemicals from their exocrine glands, including recruitment pheromone, alarm pheromone, and defensive chemicals from the poison gland (Vander Meer et al. In Press). We also determined that *P. tricuspis* phorid flies were attracted to the semiochemicals released by electrically stimulated fire ants. The objective of this work was to determine the source of the fire ant semiochemicals that attract phorid fly parasites.
Materials and Methods. We previously developed an alarm bioassay to investigate mating flight excitants (Alonso and Vander Meer 1996). In these bioassays worker ants and brood were placed in a small “Fluoned” tray where they quickly settled into a quiet clump. The air (headspace) above control and treatment samples was drawn (3 ml) into a syringe, and then 1ml of the headspace sample was carefully expressed over the quiet worker ants. Their reaction was scored on a scale of 1-4, with 1 & 2 measures of antennation (non-alarm) and 3 & 4 measures of rapid worker movement (alarm).

Automated phorid fly rearing chambers contained two rows of seven fire ant colony trays, each with workers and brood. Centrally located trays were selected as Treatments (2) and Controls (2). Prior to the experiment, pre-counts were made of resting phorid flies and those attacking in the treatment and control trays. Two, five, and 10 minutes after introduction of the Treatment and Control, the flies at rest and those attacking were again counted. Only 5 min attacking fly results are presented.

The quantity of venom alkaloids released by shaken and unshaken ants in the bioassay vial was determined by standard gas chromatographic techniques using an appropriate internal standard. Poison sacs were extirpated and crushed in hexane. The extract was diluted with hexane to allow easy application of two poison sac equivalents. This is over 100 times the amount of venom deposited by shaken ants in the alarm bioassay. The sample was applied to a small petrie dish and the solvent evaporated. Simultaneously, a control dish was treated with hexane and evaporated. The control and treatment dishes were immediately placed in the rearing boxes to determine their affect on the number of attacking phorid flies.

The quantity of recruitment pheromone released by shaken and unshaken ants in the bioassay vial was determined in terms of Dufour’s gland equivalents (DE) by an orientation bioassay. Dufour’s glands were extirpated and extracted in hexane to provide doses of 12.5 DE. This solution was used in the phorid fly attack boxes to evaluate their ability to attract phorid flies (see attack box description above).

Results and Discussion. Behavioral observations of the ants and flies suggested that the fire ant alarm pheromone may be involved in the attraction of the fly parasites to their host. Previous studies demonstrated that shaken ants release alarm pheromones that can be measured in an alarm bioassay. Shaking ants in a vial and immediately placing the vial in phorid fly-rearing boxes resulted in an increased number of attacking flies (Figure 1). While at first this appears to support the involvement of an alarm pheromone, it is possible that the shaken ants are in fact releasing additional compounds.

We analyzed vial solvent rinses for venom alkaloids and found that shaken fire ant workers release on average over 200ng of total alkaloids, which is a small amount compared to what is in a poison sac (ca. 10μg). The alkaloids represent the amount of poison sac contents that were released by the ants. Non-alkaloid compounds are in the poison sac and may elicit a behavioral response from the phorid flies. This was tested in phorid fly rearing boxes where poison sac extracts (two worker equivalents, 20 μg) were presented in a manner similar to the shaken ants.
Figure 1. The effect of the volatiles from shaken ants on the phorid fly rate of attack. Many flies are resting while others are attacking. The effect of the worker volatiles was to increase the percentage attacking flies and therefore decrease the percentage of resting flies.

The results demonstrated that even at this high concentration the poison sac contents did not elicit phorid fly attraction.

Recruitment orientation bioassays demonstrated that in one of three replicates extracts of vials, in which ants had been shaken, gave a positive bioassay result. The concentration was at the lowest detectable concentration and activity was lost with the next 1:2 dilution. Phorid fly attraction bioassays with much higher concentrations of Dufour's gland extracts (12.5 DE) demonstrated that the phorid flies were not attracted to the very small amounts of Dufour's gland products released by shaken ants (Vander Meer 1986).

All of the above indicate that of the compounds released by shaken ants, it is the alarm pheromone that is responsible for phorid fly attraction. The function of alarm pheromones dictate that they be highly volatile. Further evidence for their involvement in phorid fly attraction comes from the fact that if the shaken ant vial is left open for four minutes all activity is lost. Future research will focus on the isolation and identification of the compounds responsible for phorid fly attraction to their fire ant host.

References Cited


Bioassay Evaluation of Transgenic Strains of
Beauveria bassiana against Solenopsis invicta Buren.

Michael D. Schraeder, Harlan G. Thorvilson, and Michael San Francisco
Department of Plant and Soil Science and 1Department of Biological Sciences
Texas Tech University, Lubbock, Texas.

INTRODUCTION
A strain of Beauveria bassiana was isolated from the Mexican leaf-cutting ant (Sanchez-Pena 1992). Using transgenic procedures, a gene for benomyl-resistance was incorporated into the wild-type (1015d) B. bassiana genome. Strains 13M and 13B2B also included the genes for β-glucuronidase, a reporter or marker gene (Thorvilson et al. 2002).

OBJECTIVES
Laboratory studies were conducted to assess and compare the infective, pathogenic, and mortality properties of transformed fungal strains 13B2B and 13M to that of the original strain 1015d against Solenopsis invicta (Fig. 1).

Fig. 1. Beauveria bassiana strains used in the bioassay. From left: wild-type strain 1015d, 13B2B, 13M.

MATERIALS AND METHODS
Two transformed isolates (13B2B and 13M) and the wild-type strain (1015d) were grown in 100 X 15-mm plates on potato dextrose agar with 0.1% streptomycin (PDA+S). After mycelia produced conidia, 1-cm² plugs were cut from plates using a #6 brass plug cutter (Fig. 2). Plugs were also cut from an un-inoculated plate of PDA+S. Sterilized filter paper was placed in 75, 60 X 15-mm plates and moistened using sterilized reverse osmosis water. Fifteen plates received a

Fig. 2 (A) Plugs were cut from plates of B. bassiana for trials. (B) Solenopsis invicta in plate with fungal plug during trials.
plug of one of the fungal strains or the un-inoculated PDA+S (control-agar). The remaining 15 plates did not contain medium plugs (control).

Five laboratory colonies of *S. invicta* were selected for similar colony size and activity. Ten to eighteen ants from a colony were placed in three plates of each treatment. The experiment had three replications of each colony-treatment combination (Fig. 3). Plates were observed for two weeks, during which the numbers of living ants in each plate were recorded. Dead ants were held in moist chambers for observation of fungal growth. Two separate bioassays were completed.

Counts of surviving ants were recorded at 12-hour intervals, proportions were calculated, and proportions were arcsine-transformed for analysis of variance (critical *P*-value =0.05). Means were separated by Tukey-Kramer HSD. Graphs were constructed using untransformed percent survival data.

Fig. 3. Plates were blocked by colony during bioassays.

**RESULTS AND DISCUSSION**

Dead *S. invicta* exhibited fungal growth and conidiophore production (Fig. 4). After 72 hours, survivorship of ants in bioassay 1 (Fig. 5) treated with 1015d and 13828 was significantly less than in controls. Ants exposed to 13M had greater survival than ants in the other treatments after 96 hours.

Fig. 4. Mycelia extending and growing through exoskeletons of *S. invicta* workers.
Throughout bioassay 2, survivorship of ants in control-agar and 13M was not different (Fig. 6). Similarly, ant survivorship in control and 1015d treatments was parallel. Mortality caused by 13B2B was greater than control-agar after 60 hours; however, 13B2B mortality was rarely different from that of the control treatment.

This experiment has shown that genetically transformed strains of *B. bassiana* caused mortality of *S. invicta*. Genetic insertion in strain 13M may have interfered with pathogenicity of the fungus because survivorship was comparable to that of the control treatment.
ACKNOWLEDGEMENT

The authors acknowledge the financial support of the Texas Imported Fire Ant Research and Management Project.

LITERATURE CITED


Comparative Diagnosis of Microsporidian Infections in Fire Ant Colonies by Light Microscopy Techniques (Giemsa, Calcofluor and Trichrome Stains) and by PCR.

Yuliya Y. Sokolova*, Irina A. Isakova ‡ & James R. Fuxa*

'Department of Entomology Louisiana Agricultural Experiment Station & Louisiana State University Agricultural Center Baton Rouge, Louisiana 70803
* ‡Laboratory for Microbiological Control All-Russian Institute for Plant Protection Pobelskogo-3, St.Petersburg-Pushkin, Russia 899620

A major problem with introductions of Thelohania solenopsae in field population of Solenopsis invictae is monitoring of microsporidian infection in RIFA at release sites. The current method is microscopic examination for spores either in fresh smears under phase contrast optics or after Giemsa staining of methanol-fixed smears; this results in an unacceptably high proportion of false-negative diagnoses. The recognition of microsporidiosis as one of the opportunistic infections in AIDS patients has led to development of several alternative methods to visualize microsporidia in immunosuppressed humans and in laboratory animals. Calcofluor White and Modified Trichrome staining have become preferred methods for this purpose (Weber et al., 1992; Didier et. al., 1995; Weber, Schwartz and Deplazes, 1999). The polymerase chain reaction (PCR) also is being widely used for detecting microsporidia in human tissues, stool and body fluids; and this method is claimed to be 100 times more sensitive than light microscopy (Franzen and Müller, 1999; Weiss and Vossbrinck, 1999).

It is possible that the staining methods mentioned above, as well as PCR with conservative primers amplifying a small subunit RNA gene, could be used to detect microsporidian infections in RIFA colonies as well.

The objectives of the current work were: (1) to compare the effectiveness of phase contrast observation and Giemsa, Calcofluor, and Trichrome staining in detecting T. solenopsae in smears of tissues from ants; (2) to develop a simple, sensitive, and reliable method of detecting microsporidia in field and laboratory colonies by means of PCR, to be routinely used in conjunction with light microscopy; and (3) to compare the effectiveness of diagnosis by light microscopy and PCR.

Material and Methods

The ants from field or laboratory colonies were homogenized in sterile distilled water. Smears were prepared and examined under phase contrast microscope. Afterwards smears were air dried, fixed with absolute methanol for 5 min and stained either with Giemsa or with the Modified Trichrome stain (Weber et al., 1992). Either fresh dried or methanol fixed smears were stained by Calcofluor White M2R (Didier et al., 1995). DAPT staining was for nuclei visualization.

Thelohania solenopsae DNA isolation was performed with a Pure DNA isolation kit (Epicentre, Madison, WI) according to the manufacturer’s protocol with modifications. PCR reaction samples (25 ul) contained 10 ul of target DNA, 2.5 mM of MgCl2, 0.25 mM of dNTP; 0.4 mM each, forward and reverse primers, and 1 unit of TaqDNA polymerase (Applied BioSystems, Foster City, CA). The reaction was carried out in the thermocycler GeneAmp PCR System 970 (Applied BioSystems, Foster City, CA) under the following temperature profile:
initial denaturation at 94° C for 4 min, followed by 35 cycles of 94° C (30 sec.), 52° C (30 sec.), 72° C (1 min), and an extension step at 72° C (7 min). Spores of *Spraguea lophii* were used as a positive control.

The data were statistically treated by a Sign Test (Nonparametric statistics, STATISTICA for Windows, release 5.1, StatSoft inc. 1994-1996). For statistical analysis, the data were organized in such a way that the main variables (results of Phase contrast observations, Giemsa or Trichrome staining, or PCR tests) were placed into category 1 (success, microsporidia positive) or 0 (failure, microsporidia negative). The goal of the analysis was to compare success/failure ratios of various methods, and to assess how this ratio depends on grouping variables (approximate spore concentration [ASC] and number of ants in the sample [NA]). Calculation of ASC was based on examination of Giemsa stained slides, in which we counted the average number of spores (at 1000X) spores in 10 microscope fields. The ASCs were initially grouped into three categories: “1” - no spores in ten fields (ASC <10⁴ spores/ml); “2” - 1-3 spores per field (ASC = approximately 10⁴ spores/ml); “3” - 4-10 spores per field (ASC≥10⁵ spores/ml). The NA variable also was grouped initially into three categories: “1” - fewer than 10 ants in the sample ; “2” - 10 – 50 ants; “3” - more than 50 ants. Due to insufficient n in certain categories, the following grouping variables were used in the final analysis: ASC=1 (ASC category 1), ASC>1 (ASC categories 2 and 3 combined), NA = 1 (NA category 1), and NA>1 (NA categories 2 and 3 combined).

Results

Light microscopy. Phase contrast microscopy of fresh smears from field and laboratory colonies revealed three types of spores: octospores, *Nosema*-like spores, and megaspores, as described previously (Sokolova and Fuxa, 2001). Occasionally, earlier stages (sporoplasts, meronts, and sporonts) were identifiable as round dark bodies approximately 3-5 um in diameter. Unfortunately, detection of the microsporidium in fresh smears failed when the concentration of spores in the sample was lower than 10⁶ spores/ml. Such low resolution greatly reduces the effectiveness of this method as a diagnostic tool, because the mean concentrations of spores in the positive field samples of ants were 10³-10⁴ spores per ml.

Giemsa stain is the primary method used to detect infections in field colonies to date. At neutral pH, microsporidia stain light blue with violet nuclei. The Giemsa staining pattern is similar for all microsporidia and is described elsewhere (Undeen, 1997; Weber, Schwartz and Deplazes, 1999).

Chromotrope 2R-based Trichrome stain, modified by Weber et al. (1992), was tested on an insect system for the first time and gave promising results. The walls of all spore types are stained bright pinkish-red. Most of the octospores, measuring approximately 2.5 x 3.5 um, have a distinct pinkish-stained diagonal or equatorial stripe, similar to those described for species in the genera *Enterocitozoon* and *Encephalitozoon* (Didier et al., 1995; Weber, Schwartz and Deplazes, 1999). Immature octospores are grouped by eight inside sporophorous vesicles, the envelopes of which stain grayish. This envelope can be disrupted easily, and usually most mature octospores are observed singly in smears. Occasionally, elongated spores, representing an adjoined pair of octospores that have failed to divide, can be seen in smears. *Nosema*-like spores, measuring approximately 2.5 x 4.5 um, are elongated and often possess an intensively staining, pinkish-red posterior end. During diagnosis, they can be confused easily with free octospores. Megaspores, much larger (approximately 4 x 7 um), stain more intensively pinkish-red at both posterior and anterior ends than the other two spore types; therefore megaspores cannot be confused
with the other two types of spores. Most background debris as well as bacteria and occasional mycelia of entomopathogenic fungi counterstain a faint grayish-green. Yeast cells stain dark red and do not exhibit internal structure, which allows them to be differentiated easily from microsporidia.

**Application of fluorescent Calcofluor White stain** on methanol-fixed or unfixed smears results in turquoise (at excitation wavelength [ewl] = 395/415 nm), or blue (at ewl = 330/380 nm) fluorescence of mature and premature spores. Unfortunately, this method was unsuitable for detection of microsporidian infections in ants due to nonspecific binding with chitin debris and unstable staining of spores. For unknown reasons, only 10-40% of spores exhibited fluorescence.

**Application of DAPI** was useful for life cycle studies and for confirmation of infection, particularly by demonstration of diplokaria indicating the presence of a diplokaryotic (Nosema-like) sequence. It also helped to visualize the presence of prespore stages, which are often overlooked. DAPI stains DNA on fresh smears well, but fixation with methanol or ethanol extensively reduced the rate of fluorescence photobleaching.

**Diagnosis of microsporidian infection by Polymerase Chain Reaction (PCR).** The small subunit of the ribosomal RNA gene (ssu rRNA) has been routinely selected as the target to detect microsporidia and was explored in our research as well. Six the most common “universal” microsporidian primers (Weber, Schwartz and Deplazes, 1999; Weiss and Vossbrinck, 1999) (Table 1) were tested.

### Table 1. Conservative primers used to detect infection by *Thelohania solenopsae* (*T.s.*) in RIFA colonies after field application of the microsporidium.

<table>
<thead>
<tr>
<th>#</th>
<th>Primer pairs</th>
<th>Sequence (5’ to 3’)</th>
<th>Reference</th>
<th>Amplicon size for <em>T.s.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ssV1f</td>
<td>CAACGAGGTTGATTCTGAGCTCAC</td>
<td>Franzen &amp; Müller, 1999</td>
<td>1300 bp</td>
</tr>
<tr>
<td></td>
<td>ss1492r</td>
<td>GTTACCTTGTACGTACGAGT</td>
<td>Müller, 1999</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ssHA1f</td>
<td>GTGATTCTGCTCAGTGTA</td>
<td>Gatehouse &amp; Malone, 1999</td>
<td>1200 bp</td>
</tr>
<tr>
<td></td>
<td>ssHA5r</td>
<td>TGTAGTGCAGTGACAGCAGCA</td>
<td>Malone, 1999</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ssNAGf</td>
<td>GCCGGCCAGGAAGCCTCACCGGAGGACGCA</td>
<td>Weiss &amp; Vossbrinck, 1999</td>
<td>No amplification</td>
</tr>
<tr>
<td></td>
<td>ssNAG178r</td>
<td>ATATCGACGGGACTCACCA</td>
<td>Vossbrinck, 1999</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ssV1f</td>
<td>CAACGAGGTTGATTCTGAGCTCAC</td>
<td>Weiss &amp; Vossbrinck, 1999</td>
<td>2000 bp</td>
</tr>
<tr>
<td></td>
<td>ls580r</td>
<td>GTGTCGTTTGAAGAGCAGG</td>
<td>Vossbrinck, 1999</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ss530f</td>
<td>CGCGG(T/G)GCTGCCACGCAC</td>
<td>Franzen &amp; Müller, 1999</td>
<td>150 bp</td>
</tr>
<tr>
<td></td>
<td>ls580r</td>
<td>GTGTCGTTTGAAGAGCAGG</td>
<td>Vossbrinck, 1999</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ssV1f</td>
<td>CAACGAGGTTGATTCTGAGCTCAC</td>
<td>Weiss &amp; Vossbrinck, 1999</td>
<td>500 bp</td>
</tr>
<tr>
<td></td>
<td>ss530r</td>
<td>CGCGG(T/G)GCTGCCACGCAC</td>
<td>Vossbrinck, 1999</td>
<td></td>
</tr>
</tbody>
</table>

Detection of infection with the standard pair of primers V1f-1492r, used previously for amplifying ssRNA of *T. solenopsae* (Moser, 1995), was successful only if the concentration in the sample reached $10^6$ spore/ml, at which point the infection could be unequivocally identified by light microscopy even without staining. Only the pair V1f-530r (amplicon size approximately 500 bp) gave a stable signal at a low ($10^3$) concentration of spores, and this pair therefore was chosen to detect microsporidia in field samples (Table 2).
Table 2. Amplification efficiency of the primers.

<table>
<thead>
<tr>
<th>Primer pairs tested</th>
<th>Successful (1) / unsuccessful (0) amplification</th>
<th>Concentration of spores in the samples $10^3$</th>
<th>$10^5$</th>
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</thead>
<tbody>
<tr>
<td>Vlf-1492r</td>
<td>0 0 0</td>
<td>1 1 1</td>
<td></td>
</tr>
<tr>
<td>HA1-HA5</td>
<td>1 0 0</td>
<td>1 1 1</td>
<td></td>
</tr>
<tr>
<td>NAGf-NAGr</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td></td>
</tr>
<tr>
<td>Vlf-580r</td>
<td>0 0 0</td>
<td>1 1 0</td>
<td></td>
</tr>
<tr>
<td>530f-580r</td>
<td>0 0 0</td>
<td>1 1 0</td>
<td></td>
</tr>
<tr>
<td>Vlf-530r</td>
<td>1 1 1 1 1</td>
<td>1 1 1</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of effectiveness of Giemsa, Trichrome, and PCR tests for detection of microsporidian infection in field RIFA samples. One hundred fifty samples from two experimental sites were examined with phase contrast optics and by the Giemsa, Trichrome, or PCR tests. Thelohania solenopsae was detected by at least one of the methods in seventy-one of the samples. Sixty-one of them were evaluated by all four methods and thus were suitable for statistical analysis by the Sign Test (STATISTICA for Windows), which was applied to compare the effectiveness of the methods (Table 3).

Table 3. Rate of positive diagnosis (success/failure ratio) of microsporidian infection in ant colonies by phase-contrast microscopy, Giemsa and Trichrome staining, and PCR tests among all studied samples (explanations in the text).

<table>
<thead>
<tr>
<th>##</th>
<th>Grouping variables</th>
<th>Compared pairs</th>
<th>n</th>
<th>success/failure ratio (Mean ± SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>none</td>
<td>Phase contrast</td>
<td>61</td>
<td>0.3 ± 0.06</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Giemsa</td>
<td></td>
<td>0.6 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>none</td>
<td>Giemsa</td>
<td>61</td>
<td>0.6 ± 0.06</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichrome</td>
<td></td>
<td>0.9 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>none</td>
<td>Trichrome</td>
<td>61</td>
<td>0.9 ± 0.04</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase contrast</td>
<td></td>
<td>0.3 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>none</td>
<td>Trichrome</td>
<td>61</td>
<td>0.9 ± 0.04</td>
<td>&lt;0.012*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR</td>
<td></td>
<td>0.6 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>none</td>
<td>PCR</td>
<td>61</td>
<td>0.6 ± 0.06</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase contrast</td>
<td></td>
<td>0.3 ± 0.06</td>
<td></td>
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<tr>
<td>6.</td>
<td>none</td>
<td>Giemsa</td>
<td>61</td>
<td>0.6 ± 0.06</td>
<td>&lt;0.867</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR</td>
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<td>0.6 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>NA&gt;1</td>
<td>Giemsa</td>
<td>35</td>
<td>0.5 ± 0.08</td>
<td>&lt;0.004*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichrome</td>
<td></td>
<td>0.8 ± 0.07</td>
<td>&lt;0.342</td>
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<tr>
<td>8.</td>
<td>NA&gt;1</td>
<td>Trichrome</td>
<td>35</td>
<td>0.8 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>PCR</td>
<td></td>
<td>0.9 ± 0.04</td>
<td>&lt;0.04</td>
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<tr>
<td>9.</td>
<td>NA=1</td>
<td>Giemsa</td>
<td>26</td>
<td>0.8 ± 0.09</td>
<td>&lt;0.073</td>
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<tr>
<td></td>
<td></td>
<td>Trichrome</td>
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<td>1.0 ± 0.04</td>
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<tr>
<td>10.</td>
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<td>Giemsa</td>
<td>26</td>
<td>0.8 ± 0.09</td>
<td>&lt;0.002*</td>
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<td></td>
<td>Trichrome</td>
<td></td>
<td>0.2 ± 0.08</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>11.</td>
<td>ACS=1</td>
<td>Trichrome</td>
<td>22</td>
<td>0.6 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Giemsa</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>ACS=1; NA&gt;1</td>
<td>PCR</td>
<td>16</td>
<td>0.9 ± 0.06</td>
<td>&lt;0.077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichrome</td>
<td></td>
<td>0.6 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

Sixteen cases were identified as microsporidia-positive by phase contrast observation, 38 by Giemsa staining, 38 by PCR, and 53 by Trichrome staining. The sign test detected differences in
the rate of positive diagnosis (success/failure ratio) of microsporidian infection among the four methods. Giemsa staining was better than observation of fresh smears under phase-contrast optics; the Trichrome stain was better than the other three methods; PCR was better than phase-contrast observation of fresh smears; and the sensitivity of Giemsa staining and PCR was the same (Table 3, lines 1 - 6).

The numbers of ants and spores in samples affected the relative sensitivity of the four detection methods. When samples contained more than 10 ants (NA>1, Table 3, lines 7,8), Trichrome staining again gave better resolution than Giemsa; but PCR now had the same sensitivity as Trichrome staining. Phase contrast observation was worse than the other three methods, and resolution by Giemsa staining remained equal to PCR (data not shown). In samples with fewer than 10 ants (NA = 1), Trichrome staining was no longer more sensitive than Giemsa (Table 3, lines 9), and PCR was even less sensitive than Giemsa (Table 3, line 10). The results of comparative analyses for the other pairs remained relatively the same as in Fig.4 (data not shown). When the concentration of spores in the sample was below the sensitivity of phase contrast observation and the Giemsa test (ASC=1, Table 3, line 11), the Trichrome stain detected spores successfully PCR also did so, but only when the number of ants in the sample was more than 10 (NA>1), although its sensitivity was not significantly greater than Trichrome staining (Table 3, line 12).

**Summarizing remarks.** (i) Examination of fresh smears under phase contrast optics is not efficient and can be excluded from the diagnostic procedure. (ii) The Trichrome stain enhances diagnosis compared to the Giemsa stain and decreases the threshold concentration of spores which can be detected by at least 10 times; the Trichrome stain is therefore the method of choice for detection of microsporidia in RIFA field populations. (iii) Because spores represent only a part of the complicated life cycle of *T. solenopsae*, failure of their detection by light microscopy does not mean that the microsporidium is absent; diagnosis by PCR with V1f-530r pair primers is successful even when the concentration of spores in the samples is below the sensitivity of light microscopy.

Our results suggest a new detection procedure which should reduce the number of false-negative cases of diagnosis of microsporidiosis in field surveys of microsporidian infections in fire ants, and probably, in other insect populations. We propose staining the field samples with the Modified Trichrome stain (Weber et al., 1992) followed by PCR testing of Trichrome-negative samples.

**Acknowledgements**

We thank Arthur Richter and Igor Sokolov (Dept. of Entomology, Louisiana State University, Baton Rouge, LA) for assistance in field sampling and statistical analysis of data, and Earl Weidner (Dept. of Biological Sciences, Louisiana State University, Baton Rouge, LA) for providing spores of *Spraguea lophii*. This research was supported by a special research grant from the Louisiana Board of Regents, by USDA Southern Regional IPM grant # 99-34103-8067, and by the Texas Imported Fire Ant Research and Management Plan.

**References Cited**


Introduction: The first imported fire ant identified in the United States was the black imported fire ant *Solenopsis saevissima richteri* Forel (Loding 1929). Creighton (1930) estimated the arrival of *S. richteri* to be around 1918 through the port of Mobile, AL. A second species, the red imported fire ant *Solenopsis invicta* Buren also arrived through the port of Mobile, AL sometime between 1933 and 1945 (Lennartz 1973). The fact that there were two separate species present in the United States was not fully recognized until Buren (1972) revised the taxonomy for the imported fire ants. More recently, it has been discovered that a hybrid form of the two species also exists (Vander Meer & Lofgren 1985; Diffie et al. 1988).

Information and data relative to the range expansion of the imported fire ants, *Solenopsis richteri* Forel and *S. invicta* Buren, is scattered throughout the literature. Several authors have examined range expansion through 1953 to 1974 (Culpepper 1953; Wilson & Brown 1957; George 1958; Adkins 1970; Buren et al. 1974). More recent authors have briefly reviewed range expansion before discussing the history of imported fire ant control (Lofgren 1986) or quarantine (Lockley & Collins 1990). This document is not intended to speculate on possible future range expansion, but to compile known information on range expansion of imported fire ants into one source.

Materials and Methods: In order to accomplish our objective, several ground rules were established.

1. The overall expansion of all imported fire ants was established; expansion of individual species or their hybrid was not differentiated.

2. Over the years, the Federal Imported Fire Ant Quarantine (Code of Federal Regulations, Title 7, Section 301.81 – hereafter cited as CFR) has listed the affected areas in a variety of ways: regulated areas, generally infested areas, eradication areas, suppressive areas and quarantined areas. For our purposes, we consider any county/parish listed in the CFR as an infested area.

3. Pockets of infestations may be present in my given year outside the area listed in the CFR for that year. As these areas are not cited in any consistent manner, they were not included.

4. In determining the total number of acres infested per year, total acreage infested per county/parish was extrapolated from the literature for 1918-1953. From 1958-present, all data came from the annual CFR, and total acreage of an infested county/parish was used regardless of whether the county/parish was entirely or partially infested (except in CA...
where fraction of county used for partially infested). County/parish acreage was obtained form Rand McNally (1993).

5. As stated above, form 1958-present, the CFR was used as the sole reference for range expansion of the imported fire ant. However, in some years, no additions were made to the manual. In those years it is unknown if new areas meeting the criteria necessary for inclusion into the federal quarantine were not found, or if no surveys were conducted due to lack of manpower or funds.

Discussion: From its small toehold in Mobile, AL in 1918, imported fire ants spread over 62,448,000 acres into all or part of 141 counties/parishes in eight states by 1958. In 2000, the pest had expanded its range to a total of +320,000,000 acres in all or part of 749 counties/parishes in 11 southern states, 1 county in New Mexico, 3 counties in California, and Puerto Rico. The most current Federal Quarantine map is at: www.aphis.usda.gov/PPQ/maps/fireant.pdf. References cited available upon request.

Infested Counties – ca 1939
Adkins 1970*

4,820,800 acres

* Did not differentiate between whole and partial
1975 Imported Fire Ant Quarantine
CFR 301.81

2001 Imported Fire Ant Quarantine
CFR 301.81
EVALUATION OF "ORGANIC" PRODUCTS AND HOME REMEDIES TO ELIMINATE RED IMPORTED FIRE ANT COLONIES

Bastiaan M. Drees and Paul R. Nester

Texas Cooperative Extension, TX A&M University System

Interest in home remedies and "organic" treatments for the red imported fire ant, Solenopsis invicta Buren, remains high. A number of home remedies such as soap solutions (Roberts 1987) and instant grits (Garrett, 1993) have been proposed or supported with anecdotal observations. However, few have actually been scientifically evaluated in formal field trials. Some products (Erath Earth Orange Oil, Erath Earth Gathering and Holding co., Hico, TX and Ridants®, CedarCide Industries, Inc., Spring, TX) are not pesticides registered by the Environmental Protection Agency (EPA), but are sold as "elixirs relating to plants and insects" or are promoted to control fire ants while claiming to be exempt from registration. The 25(b) clause of the Fungicide, Insecticide Rodenticide Act (FIFRA) allows products containing certain food grade active ingredients and inert ingredients to be exempt from FIFRA, which requires EPA registration provided they are approved for sale in Texas by the Texas Department of Agriculture (TDA) and have effectiveness proven through scientific evaluations (efficacy trials).

The series of trials reported herein were conducted to generate field data to align product or treatment performance with consumer expectations. Results are not intended to provide a basis for the endorsement or recommendation for use by the Texas Imported Fire Ant Research & Management Project, the Texas Cooperative Extension or the Texas Agricultural Experiment Station at The Texas A&M University System.

Materials and Methods

Trials were conducted on the Texas A&M University campus on grounds located near the George Bush Presidential Library. This area is regularly mowed and contained predominantly Bermuda turf grass. Plots were established by locating, and marking with field flags, sets of 10 red imported fire ant (henceforth referred to as the fire ant) mounds in areas of roughly equal widths and varying in length depending on fire ant mound density. Plot areas were calculated and arrayed from smallest to largest plot. Blocks of treatments were then established with one block (replication) containing the smallest plots, one block with medium-sized plots and one with the largest plots. This method accommodates fire ant colony movement: through the course of the trial. Within each block, treatments were randomly assigned so that each trial's treatments were replicated three times.

All treatments (Appendix 1) were applied from 1 gal jugs through a colander to break the flow into a gentle sprinkle to simulate a garden sprinkler. Additional mounds detected in plots at the time of treatment were marked with different colored flags to eliminate them from further evaluation.

Fire ant mounds were assessed using the minimal disturbance method, whereby mounds were disturbed with a field flag or shovel. When 12 or more fire ants emerged from the disturbed
mound in a defensive reaction, the mound was considered to contain a fire ant colony. This method was used to establish plots and to evaluate treatments periodically following application of treatments. At the later post-treatment evaluations, an effort was made to assess other active fire ant mounds within treatment plots, thereby providing data on “new” or “satellite” mounds which result from colony movement. However, due to the appearance of many “new” mounds in plots and abandonment of untreated controls in both dry and water-drenched check plots, new mounds were recorded either for occurring within a 5 ft. radius of the treated and marked mound or occurring within the plot. The new mounds closer to the treated mounds were considered more likely to represent “satellite” mounds caused by fire ant colony migration or “shattering” of colonies whereby the treated colony split into two or more “new” colonies.

**Trial 1.** This trial was established on September 26, 2001 ranging in size from 5,320 to 14,570 sq. ft. Treatments were applied Sept. 27 in the afternoon, when the weather was clear and the high temperature reached 82 degrees F. Treatments included:

1) Untreated dry check mounds
2) Water only - 1 gallon water per mound
3) Liquid dishwashing detergent - 2 fl oz (4 Tbsp) Dawn
4) Liquid dishwashing detergent plus orange oil - 1 fl oz (2 Tbsp) Dawn plus 1 fl oz (2 Tbsp) Erath Earth Orange Oil
5) Citrex® Fire Ant Killer - below labeled rate application of 3 fl oz/gallon (the new, low rate currently pending labeling is 5 fl oz/gal, pers. com. Craig Gant)

**Trial 2.** Plots were established on September 26, 2001 with additional treatment plots established on Sept. 28 and Oct. 4

1) Untreated dry check mounds (from Trial 1). Treatments included:
2) Instant grits (Quaker® Instant Grits Original, The Quaker Oats Company, P. O. Box 049003, Chicago, IL 60604-9003) - 0.5 cup per mound, minimum (three plots treated Sept. 27 (I), 28 (II), and Oct. 1 (III), respectively).

**Trial 3.** Plots were established October 3 and 4, 2001 and treatments were applied from 10:00 a.m. to 1:00 p.m. on Oct. 4. Temperature was 86 degrees F and weather was clear. Treatments included:

1. Water, only - 1 gallon water per mound
2. Ridants® - 1 gallon Ready-To-Use drench per mound
3. CedarCide Pet, Horse & Livestock Concentrate - 1 Tbsp plus 1 tsp per gallon per mound.

In addition, five fire ant mounds were treated with TFA Super-Kill™ Fire Ant Eliminator using a sample and directions provided by the manufacturer. This treatment was not considered part of Trial 3 although mounds were drenched Oct. 4, 2001.

Data from all Trials were analyzed using analysis of variance (ANOVA) and means were separated using the Duncan’s Multiple Range Test at the 5 percent level of probability (Microstat, Ecosoft Inc., Indianapolis, IN).
Results

No significant rainfall occurred until after October 5, with weather being mild (daytime high temperatures in the mid-80 and nights in the 60 degree F range) and dry. Soil moisture was fairly dry. Thereafter, rains saturated soils and brought fire ants to the surface and dramatically increasing the number of fire ant mounds in plots. In additions, many colonies in dry and water-drench control (check) plots relocated. As a result, we began recording fire ant mounds within a 5 ft radius circle of the originally treated mound.

Trial 1. Three days after treatment, all mound drench treatments, including a water-only treatment, significantly reduced numbers of active fire ant mounds relative to untreated (dry) control/check mound plot means (Table 1). The soap and water only treatments provided similar results as did the Citrex® and citrus oil plus soap treatments, although the latter provided significantly better control. From 8 to 29 days after treatment, Citrex®, soap and citrus oil plus soap treatments provided significant reductions of treated and marked fire ant mounds relative to both water-only and dry untreated plot means. Furthermore, when accounting for colony movement within a 5 ft radius of the treated mound site, the Citrex® and citrus oil and soap treatments provided significant reductions of mound numbers. Due to the appearance of numerous “new” mounds within plots, no treatment provided significant reductions of fire ant mounds within plots 15 and 29 days following treatment.

Trial 2. Quaker® Instant Grits Original, applied at 0.5 cup per fire ant mound resulted in no significant reductions relative to untreated control (dry check) plot mean mound numbers over the 29 day duration of this trial (Table 2).

Trial 3. Although the number of treated and marked fire ant mounds drenched with CedarCide Pet, Horse & Livestock Concentrate were numerically reduced 1 day after treatment, the reduction was not significant (Table 3). Fire ant mounds treated with Ridants® were still active although the number of fire ants appeared to be dramatically reduced. Three days after treatment, the cedar oil containing products had significantly reduced active fire ant mound numbers relative to water only mound drench untreated control (check) plot means. Thereafter, new fire ant mounds appearing in plots eliminated significant differences between treatment plot fire ant mound numbers through the 29 day duration of this trial.

Interestingly, on the 15th day after treatment (October 19), the treated and marked mound site plot means for the two cedar oil products were significantly lower than untreated check plot means. However, then the number of fire ant mounds within a 5 ft radius of the marked site was analyzed, more fire ant mounds were found in cedar oil drenched plots, with significantly more in plots treated with CedarCide Pet, Horse & Livestock Concentrate. These data imply that cedar oil products are, in fact, acting primarily as insect repellent products - not contact insecticides. Treated fire ant colonies not only relocated to nearby sites, but they also split into several fire ant colonies (a phenomenon also called shattering). This site is suspected of harboring the multiple queen (polygynous) form of the fire ant which are capable of colony splitting because each mound contains more than one reproductively active queen ant.
Discussion

September and October are the rainiest months of the year in College Station, Texas (Figure 1). At this time fire ant colonies that have been dwelling deeper in the soil and not producing visible mounds begin to work towards the surface seeking warmer soil or escaping saturated soils from recent rains. Freshly-built mounds are evidently not stable in their location and fire ant colonies frequently move, evidently in search of better nesting sites or escaping any type of irregularity, i.e., disturbance, disease, predators, etc. Their mounds are not well constructed with “honey-comb” galleries characteristically built by ant species inclined to stay in one location for longer periods of time. However, these more transient fire ant colonies build mounds of freshly-dug earth piled on top of turf grass. Thus, the strategy of locating and treating visible mounds misses the colonies dwelling underneath the soil surface, and treated colonies readily abandon mounds and produce new ones in the near vicinity.

Use of fire ant mound numbers as an indicator of fire ant populations has been brought into question, in part, because of the experience gained by conducting these three Trials. Perhaps it is better to think of fire ant mounds as bubbles in boiling water or as glass balls in a Galileo liquid thermometer which rise to the surface under specific conditions. However, as relative indicators of ant fire ant population levels between treatments at a given point in time, ant fire ant mound numbers can still be an appropriate measure.

Trial 1. Plant oils, as those contained in citrus peels (d-limonene), pine (turpentine) and cedar are known to contain ingredients toxic to some insects. Soaps contain surfactants that can cause direct insect mortality by suffocating or drowning pests and can also act as emulsifiers to allow oils to be mixed into water. Soaps also cut through the wax-covered insect exoskeleton allowing active ingredients in oils to penetrate and act to kill cells (cytotoxicants). This effect can be seen in the results of Trial 1 where the 2 fl oz rate of liquid dishwashing detergent eliminated fewer active ant mounds than did the treatment containing half that amount of detergent (1 fl oz) plus 1 fl oz orange oil.

Plant oil and soap drench treatments must directly contact the target pests in order to eliminate them. In these trials, one gallon of solution was used to treat each fire ant mound, regardless of its size. Larger fire ant mounds were less likely to have all fire ants eliminated by any of the liquid drench treatments, although the number of worker fire ants emerging from minimally disturbed mounds were far fewer in number following treatments than in water drenched or untreated (dry check) mounds. Thus, the evaluation of active or non-active mounds is a fairly stringent measure of product performance requiring virtually all fire ant activity in treated mounds to be eliminated before it was determined to be inactive.

Trial 2. Use of instant grits has been reported to eliminate fire ant colonies by users for a long time, and some people are adamant about their observations. However, the results from use of any fire ant mound treatment is extremely difficult to assess without conducting a replicated, statistically analyzable trial. The results of these trials have pointed to some of these difficulties. In Trial 1, even application of one gallon of tap water drenched on fire ant mounds resulted in some numerical reduction of mounds at treated sites compared to the dry untreated control (check) plot fire ant mound means (Table 1). This field trial provides further documentation that this home remedy is ineffective as a fire ant treatment.
Trial 3. Ridants, applied as directed, appears to repel fire ant colonies from treated sites within three days of application. However, colonies do not appear to be eliminated. When a higher concentrated cedar oil product (CedarCide Pet, Horse & Livestock Concentrate - not currently promoted for fire ant control) was used, treated fire ant mounds split as well as relocated, resulting in more fire ant mounds near the one treated initially. Use of this product for managing fire ants should be carefully thought through. As a repellent, this product may be useful for rendering certain locations, such as potting media, temporarily fire ant free. However, resulting fire ant colony relocation and splitting following treatment makes use of these treatments unlikely for use to reduce fire ant populations.

As with any field trial to assess the effectiveness (efficacy) of fire ant control products, confidence in the results presented here can be improved by conducting additional replicated trials at different locations and times of the year.

Literature cited


Acknowledgments

The authors are grateful for permission to use this site for conducting these evaluation, with permission provided by Thomas W. Dew, Jr., Superintendent for Landscape and Pavements Maintenance with the Physical Plant Department Facilities Maintenance & Renovation of Texas A&M University (979/845-5511; FAX: 979/458-0456; e-mail: t-dew@tamu.edu, with cooperation from Tommy Palmos and Mike Faust. Products for evaluations were graciously provided by Dave L. Glassel, CedarCide Industries, Inc and Craig Gant, EnviroSafe Labs, LLC.
Appendix 1. Treatment product information

New Fast Acting Formula Ultra Dawn Original Scent (1.48 l (1.56 qt) or 50 fl oz. container for $3.58). Dawn contains biodegradable anionic and nonionic surfactants and no phosphate. (Proctor & Gamble, Cincinnati, OH 45202; 800/725-3295)

Erath Earth Orange Oil ($13.95/1 qt (0.95 l); 1 to 2 oz. per gal. for foliar spray or 6 to 8 oz. per gal soil drench application rates). Cold press orange peel extract is one of the best oils for use in the preparation of organic elixirs relating to plants and insects. (Erath Earth Gathering and Holding co., Rt. 2, Box 11, Hico, TX 76457)

Citrex™ Fire Ant Killer (d-limonene (7.2 lbs./gal.) 78.2%; 32 fl. oz.; 8 fl. oz./gal/mound application rate). Do not disturb mound prior to applying mixture. Mix with water according to the dilution chart. Apply the mixture in the early morning before ants become active. Apply the mixture in a circular motion starting at the base of the mound and continuing to the top of the mound. Make sure fire ant mound is completely saturated. Mix only enough to use at one time. Do not store mixture overnight; avoid contact with eyes or clothing. (WARNING, EPA Reg. No. 72244-1-72440). (EnviroSafe Labs, LLC, 210 North Loop 336 East, Conroe, TX 77301; www.envirosafelabs.com)

Ridants® (0.004 cedar oil; retail price: $4.99/gal ready-to-use; 1 gal. (3.787 l/mound application rate). Carefully wipe the ant mound with a long handled shovel or rake, exposing the ant eggs. This is best done in the heat of the day. Drench the mound and surrounding area with CedarCide Ridants. For greater coverage dilute 1 gallon of Ridants with 4 gallons of water (optional). To insure results, drench exposed tunnels 12 to 24 hours later. To prevent remigration of ants or other insects apply CedarCide Pesticide Granules in desired areas. For economical treatment of multi-acre infestations. soak 1 part CedarCide granules with 3 parts water for a 48 hour period, use the unpurified solution in the same manner described above. This product is classified as a minimal risk pesticide by the Environmental Protection Agency of the U.S.A. ruling 40CFR 152.25 not intended for tick control. Promotional literature statements - “Put an end to fire ants without danger to humans or animals. Apply this liquid direct or dilute it for larger mounds. Ants digest the special formula while salivating the soaked soil, a process used to repair their destroyed mound. As the worker ants become ill, reinforcements are called upon until all the ants are dead. CedarCide granules in your yard will repel ants and eliminate infestations. For large colonies of ants, we suggest brewing a batch of your own liquid from granules. Directions on bag. (Dave L. Glassel, Founder and CEO, CedarCide Industries, Inc., P. O. Box 549 Spring, TX 7783; 800/842-1464, www.cedarcide.com)

CedarCide Chemical Free Pure Cedar Oil Pet, Horse & Livestock Concentrate (50% cedar oil; 1 qt container). Mix ½ pint for 25 gallons of water; dilute solutions 400 to 1 for gardening use; safe to use directly on fruit and vegetables; repels numerous insects including ants; CAUTION - avoid contact with eyes; do not take internally; wash hands with soap and water after use; keep out of reach of children. CedarCide 66/33 concentrations are approved as a minimum risk pesticide by the EPA 40CFR 152.2.B. (Dave L. Glassel, Founder and CEO, CedarCide Industries, Inc., P. O. Box 549 Spring, TX 7783; 800/842-1464, www.cedarcide.com)

TFA Super-Kill™ Fire Ant Eliminator (pine oil 89%; 32 fl. Oz; 5 ounces (5/8/ cup) per gallon per mound application rate) Shake well before using; pour mixture onto the ant mound starting at the perimeter and continuing in a circular motion to center of the mound; do not disturb the mound before treatment; apply product gently to avoid disturbing the ants; use one gallon of mixture per ant mound; confine mixture to the area of the ant mound in applications to lawns; product may cause browning of, or kill, grass around edges of ant mounds; for best results, use during cooler parts of the day, early to mid-morning, and not during prolonged hot and dry conditions. Precautionary statements - corrosive, causes eye damage; harmful if swallowed; causes skin irritation; do not get in eyes, on skin or clothing; wear goggles or face shield and rubber gloves when handling; wash thoroughly with soap and water after handling; do not store near heat or open flame. (TFA Products, Inc., Houston, TX 77079, DANGER, EPA Reg. No. 70072-1)
Table 1. Red imported fire ant mound numbers per 10 mound plot and per plot following September 27, 2001 application of an "organic" product and selected home remedy ant mound drench treatments, Brazos Co., TX.

<table>
<thead>
<tr>
<th>Treatment (Sept. 27)</th>
<th>Area (sq. ft.)</th>
<th>Sept. 30 3 day</th>
<th>Oct. 5 8 days</th>
<th>Oct. 12b 15 days</th>
<th>Nov. 2b 29 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check (1 gal. water)</td>
<td>8,304.0</td>
<td>7.7b</td>
<td>8.7a</td>
<td>6.3</td>
<td>6.7a</td>
</tr>
<tr>
<td>Citrex</td>
<td>8,565.0</td>
<td>2.0c</td>
<td>3.3b</td>
<td>1.3b</td>
<td>1.7b</td>
</tr>
<tr>
<td>Soap</td>
<td>9,437.3</td>
<td>6.3b</td>
<td>5.0b</td>
<td>1.0b</td>
<td>2.7b</td>
</tr>
<tr>
<td>Citrus Oil plus Soap</td>
<td>8,557.3</td>
<td>1.0c</td>
<td>5.0b</td>
<td>1.3b</td>
<td>1.3b</td>
</tr>
<tr>
<td>Dry Ck</td>
<td>15,082</td>
<td>9.7a</td>
<td>10.0a</td>
<td>5.3a</td>
<td>7.0a</td>
</tr>
</tbody>
</table>

|               |               |               |              |                 |                |
| Mean Square   | 41.33         | 35.27         | 11.40        | 22.77           | 21.50          |
| F ratio       | 70.86         | 15.91         | 7.125        | 18.71           | 7.049          |
| Probability   | 0.0000        | 0.0007        | 0.0095       | 0.0004          | 0.0098         |
| SSD 5%        | 1.553         | 3.028         | 2.572        | 4.101           | 26.174         |
| d. f. = 4; n = 3 |               |               |              |                 |                |

Means in columns followed by the same letter are not significantly different using ANOVA and separated using the Duncan's Multiple Range Test at the 5% level; NS = not significant (Microstat).

Number of active mounds out of 10 marked and treated; indented - including the number of active mounds within 5 ft radius of treated mound considered to be "satellite" mounds; and, double indented - total number of active mounds per plot.
Table 2. Red imported fire ant mound numbers per 10 mound plot and per plot following September 28 through October 1, 2001 application of 0.5 cup instant grits to three of six plots, Brazos Co., TX.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oct. 5</th>
<th>Oct. 12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Oct. 22&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Ck</td>
<td>10.0</td>
<td>5.3</td>
<td>8.0</td>
</tr>
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<td></td>
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<th>Oct. 12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Oct. 22&lt;sup&gt;b&lt;/sup&gt;</th>
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SSD 5%<sup>a</sup>

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<th>Oct. 12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Oct. 22&lt;sup&gt;b&lt;/sup&gt;</th>
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d. f. = 1; n = 3

<sup>a</sup> Means in columns followed by the same letter are not significantly different using ANOVA and separated using the Duncan’s Multiple Range Test at the 5% level; NS = not significant (Microstat).

<sup>b</sup> Number of active mounds out of 10 marked and treated; indented - including the number of active mounds within 5 ft radius of treated mound considered to be “satellite” mounds; and, double indented - total number of active mounds per plot.
Table 3. Red imported fire ant mound numbers per 10 mound plot and per plot following October 4, 2001 application of cedar oil-containing liquid drench products, Brazon Co., TX.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Area (Oct. 4) (sq. ft.)</th>
<th>Oct. 5 (Oct. 4)</th>
<th>Oct. 7 (3 days)</th>
<th>Oct. 12&lt;sup&gt;b&lt;/sup&gt; (8 days)</th>
<th>Oct. 19&lt;sup&gt;b&lt;/sup&gt; (15 days)</th>
<th>Nov. 2&lt;sup&gt;b&lt;/sup&gt; (29 days)</th>
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<td>4.3a</td>
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<td></td>
<td>7.0</td>
<td>8.7b</td>
<td>10.3</td>
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<td>Ridants</td>
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<td>27.3</td>
<td>37.7</td>
<td>35.7</td>
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Mean Square
- 14.78 -- 10.11 11.44 11.44 11.44
F ratio
- 11.57 -- 13.00 5.71 5.71
Probability
NS 0.0217 NS 0.0178 0.0726 NS 0.0674 NS NS NS
SSD 5%
7.59 2.61 2.11 2.11 2.04 3.36 2.04 2.04 3.36 6.79 5.50 10.12 6.79 5.50 10.12 24.33 22.81 29.77

d. f. = 2; n = 3

<sup>a</sup> Means in columns followed by the same letter are not significantly different using ANOVA and separated using the Duncan’s Multiple Range Test at the 5% level; NS = not significant (Microstat).

<sup>b</sup> Number of active mounds out of 10 marked and treated; indented - including the number of active mounds within 5 ft radius of treated mound considered to be “satellite” mounds; and, double indented - total number of active mounds per plot.
Figure 1. Daily mean maximum and minimum temperatures and daily mean precipitation, College Station, TX.
Establishment of the Phorid Fly, *Pseudacteon curvatus*, in Alabama for Biological Control of Imported Fire Ants

L. C. Graham¹, S. D. Porter², V. E. Bertagnolli¹, H. D. Dorough³, and A. T. Kelley⁴

¹Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849-5413
²USDA-ARS Center for Medical and Veterinary Entomology, Gainesville, Florida 32604
³Auburn University, Alabama Cooperative Extension System, Talladega, Alabama 35160
⁴Department of Entomology and Plant Pathology, Mississippi State University, Starkville, Mississippi 39762-9775

Introduction

When fire ants were introduced into Alabama in the early 1900's, almost all of their natural enemies were left behind in South America (Jouvenaz, 1990). As a result, fire ant densities are much higher in Alabama than they are in South America (Porter et al., 1997). Two species of imported fire ants occur in Alabama. The red imported fire ant, *Solenopsis invicta*, is located primarily in the southern portion of the state. The black imported fire ant, *Solenopsis richteri*, is now only found in a small population located in northwest Alabama and northeast Mississippi. A hybrid of the two species populates the northern part of Alabama. One group of natural enemies that have shown some promise in the battle against fire ants are phorid flies in the genus *Pseudacteon* (Porter, 2000). These flies have the unusual habit of decapitating fire ant workers. *Pseudacteon tricuspis* was released into *S. invicta* populations in southern Alabama and has been established since 1999. *Pseudacteon curvatus* was released into the hybrid fire ant population located in Talladega County in 2000.

*Photo courtesy of Sanford Porter*
Materials and Methods

Fire ants were collected from a farm in Talladega County and were shipped to Sanford Porter in Gainesville, FL. The collected ants were identified as hybrids of *S. invicta* and *S. richteri* by their color and cuticular hydrocarbon patterns. Once confirmation of a hybrid population was received, mounds at the site were individually marked with numbered flags. Approximately five grams of workers were collected from a mound and placed into a plastic container labeled to correspond to the mound number. Beginning May 4, 2000, workers were collected from either six or thirteen mounds on a single date, depending on the number of shipping containers available. The plastic containers with the workers inside were placed into a cooler with an ice pack and shipped overnight to S. Porter. The workers were exposed to a laboratory colony of *P. curvatus* for 2-3 days. The workers were then repackaged and shipped overnight back to our lab or to Henry Dorough in Talladega County.

Exposed workers were returned to the field within 24 hours of receipt and were placed into the mound from which they were originally removed. This process was repeated a total of five times. Workers were collected from 6 mounds on 4 and 17 May and from 13 mounds on 8 and 23 May, for a total of 38 mounds.

Results and Discussion

The search for a resident population of *P. curvatus* was initiated on June 16, 2000. The first siting of *P. curvatus* occurred on Aug. 10. Three flies were captured on Aug. 14 and were positively identified as *P. curvatus*. Flies were observed at the site again on Aug. 28 and Sep. 14 and 26.

In 2001, flies were found in May and July. On Aug. 16, several areas around the release site were sampled. No flies were found to the south of the release site. However, the phorids were found in mounds approximately 1.1 kilometers north of the release site. This is the first successful establishment of *Pseudacteon curvatus* in the United States.

References Cited


THE SCIENCE OF FIRE ANTS
Kathy S. McLean and Fudd Graham

Dept. of Entomology and Plant Pathology
301 Funchess Hall, Auburn University, Auburn, AL 36849-5413

Introduction

The Science of Fire Ants project is based on the belief that hands-on experiences are most effective in learning and must occur in a relevant contextual structure. The National Science Education Standards are used by all science teachers across the nation. The project was conceived using these standards as a guide. We emphasize the importance of the students' background and the available resources in the local environment in planning curricula. The Science of Fire Ants demonstrates the availability of easily accessible, inexpensive teaching resources which can be found in their own back yard. This project utilized the students' inherent familiarity with the world around them as a basis for constructing scientific knowledge and thought. The close relationship of fire ants to the lives of the students helps motivate typically reluctant students and makes science a relevant part of their education.

Objectives

Our objectives of this educational project were: 1) to improve classroom instruction in science through the use of available resources in the environment, 2) to enhance teacher knowledge of science content and data analysis, and 3) to increase the general knowledge-base of fire ants in Alabama middle school science teachers.

The Workshop

Middle school science teacher workshops were conducted at Auburn University in June of 2000 and 2001. A total of 150 Alabama teachers and 30 undergraduate education majors from thirty-five counties across Alabama (see map) participated in the workshops. Each two hour workshop models hands-on fire ant science projects the teachers can take back to their classrooms to increase their students' knowledge-base of science, math, and fire ants.
Hands-on methodologies were incorporated into the fire ant science experiments. These methodologies included exploring current knowledge, investigating ideas, hypothesizing theories, conducting experiments, verifying concepts and clarifying misconceptions about fire ants.

Activities followed the Learning Cycle Model of instruction. We began with an activity designed to determine participants' current conception of fire ants. Then we determined what new knowledge they desired to learn. Experiments were constructed to explore their hypotheses. Assessment was conducted throughout the experiments through use of scientific reasoning and processing of data collected during experimentation. This constructivist approach allowed participants to work in partnerships and to design experiments to answer questions raised by the group. Group activities involved creative thinking and reasoning with a strong emphasis on experimental design. The interrelationship of math and science was emphasized in records, calculations, and graphs produced as a result of these activities that required constant measurement and observations.

A primary emphasis of the fire ant project included monitoring all stages of metamorphosis of fire ants, allowing the teachers to observe and chart the fire ant life cycle.

All fire ant projects demonstrate the scientific hypothesis procedure and require observations, data collection, data analysis and summary of results.
Assessment of the workshops was conducted by the teachers’ response to the following evaluation form:

Please give honest and thoughtful answers to the following questions using the 1 -5 rating scale with 1 = Very low; 2 = Low; 3 = Medium; 4 = High and 5 = Very High.

Average ratings of 150 teacher participants in June 2000 and 2001.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Question</th>
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</thead>
<tbody>
<tr>
<td>2.75</td>
<td>What was your interest in this workshop before you came?</td>
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<tr>
<td>4.49</td>
<td>What was the overall effectiveness of the fire ant workshop?</td>
</tr>
<tr>
<td>4.38</td>
<td>Did we make clear the goals of the workshop?</td>
</tr>
<tr>
<td>4.57</td>
<td>Did we introduce the subject matter effectively?</td>
</tr>
<tr>
<td>4.68</td>
<td>Did we stimulate you to think more deeply about fire ants?</td>
</tr>
<tr>
<td>4.32</td>
<td>Did we comment on your work in a way to help you learn?</td>
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<tr>
<td>4.57</td>
<td>Did we increase your desire to learn about fire ants?</td>
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<tr>
<td>4.64</td>
<td>Was the workshop well prepared?</td>
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<tr>
<td>4.34</td>
<td>How much did you learn in this workshop?</td>
</tr>
<tr>
<td>4.36</td>
<td>Will you include these demonstrations in your class?</td>
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</tbody>
</table>
ANNUAL LOSSES CAUSED BY RED IMPORTED FIRE ANTS TO HOUSEHOLDS IN THE SOUTHERN U.S.

Lynne C. Thompson and Suzanne Wiley

1 Arkansas Forest Resources Center, School of Forest Resources, University of Arkansas - Monticello, Monticello, AR, 71656-3468  
email: thompson@uamont.edu

2 University of Arkansas, Cooperative Extension Service, Arkansas Forest Resources Center, Monticello, AR 71656-3468

Abstract For most American families, their household and its land base are a focal point of their lives. The people living on these parcels of land decide what they want to spend to maintain their quality of life. Because red imported fire ants (RIFA) can influence this quality of life, decisions about reducing RIFA populations to tolerable levels would be made at the household level, based on the losses perceived by the occupants of that household. Thus, knowing two kinds of information should allow one to estimate household losses from RIFA over large areas: 1) number of households, and 2) mean losses per household induced by the RIFA. A recent study from South Carolina (Miller et al. 2000) provided excellent RIFA loss information and the 2000 Census provided a definitive count of the number of households. When united, they showed that RIFA causes annual losses of $2.5 billion (± 0.5 billion) across 11 southern states that are totally or partially infested. These losses are likely to be concentrated in densely populated areas. Because RIFA losses are induced in many other sectors of the economy (like cities, schools, golf courses, businesses, airports, utilities, agriculture, and many others), overall losses are likely to be much higher. Until losses in these sectors can be quantified, overall annual losses will remain obscure.

Introduction

Red imported fire ants (RIFA) [Solenopsis invicta Buren] have been in the U.S. for more than 80 years (Callcott & Collins 1996). During this time they have caused many problems for humans and the things humans desire (Vinson 1997). Many studies have been done to assess the economic effects that RIFA inflict directly and indirectly on humans (e.g., Ervin & Tennant 1990, Jemel & Hugh-Jones 1993). Most of these studies are of small scale, assessing the effects in experimental plots or specific situations (e.g., Banks et al. 1990, Schulz 1991, Semenov et al. 1997, Anon. 1999). Few studies have tried to assess the effects of RIFA over larger areas like states or regions. In 1995, Thompson et al. expanded the results obtained from a mail survey conducted in southern Arkansas (Thompson and Jones 1994) into 9 heavily infested southern states. This report suggested that RIFA caused about $2 billion in annual losses. Although the legitimacy of this economic expansion of local results to regional levels was questioned by economists, the results spurred other studies designed by economists to assess the "real" effects of RIFA. Two of these studies were completed in 2000. Salin et al. (2000) reported on RIFA losses in five of the largest metropolitan areas of Texas. They found that annual household losses totaled more than $526 million. Salin et al. (2000) also assessed the losses for cities, school, and golf courses to get total annual losses in all categories of
losses for cities, school, and golf courses to get total annual losses in all categories of more than $581 million for these 5 metroplexes. More recently, Miller et al. (2000) used a telephone survey in South Carolina to assess state-wide losses. They found that annual RIFA losses averaged $80.37 per household, but when labor to apply pesticides and remediate problems was included, average household losses increased to $100.93 (95% CI ± $17.05).

With these excellent studies in print, it seemed reasonable to reassess the southwide losses caused by RIFA using the new statistics, the methods of Thompson et al. (1995), and the results of the 2000 census. Thus, the objectives of this study are to expand the mean losses per household reported for South Carolina across the RIFA infested states of the southern U.S.

Methods
Thompson (1995) reasoned that RIFA are typically controlled at the household level. That is, the people living on specific parcels of land decide what they want to spend to maintain their quality of life. Because RIFA can influence the quality of life, decisions about reducing RIFA populations to tolerable levels would be made at the household level, based on the losses perceived by the occupants of that household. Thus, knowing two kinds of information should allow one to estimate losses from RIFA over large areas: 1) number of households, and 2) mean losses per household induced by the RIFA.

The number of households in individual counties is estimated most years by the U.S. Census Bureau. Fortunately, the 2000 census provides the best estimate to date of households because it is based on an actual count. The two recent RIFA impact studies (Salin et al. 2000, Miller et al. 2000) provided the second bit of critical information. Making calculations simply requires determining the number of households in infested counties and multiplying this number by the mean losses per household.

A map of infested counties is provided annually by APHIS. We used the 2000 map (USDA APHIS 2000) to determine infested counties and states (Figure 1). For ease of calculations, we considered all infested counties, whether entirely or partially quarantined by APHIS, to be totally infested.

The household data for all states and counties in the southern U.S. (US Census 2000) was moved into an ArcView GIS data base and uninfested counties removed. Total households per state were summed and then moved into a Quattro Pro spreadsheet for the calculations. Because the results of Miller et al. (2000) were based on a nice mix of both rural and urban situations, they made our calculations much simpler. We used the mean losses per household with labor costs from Miller et al. (2000). We also used Miller et al.'s 95% confidence intervals to show variation.

Results & Discussion
Losses for infested southern states are shown in Table 1. Although the total estimate of $2.5 billion (95% CI ± 0.5 billion) is coarse, it provides a vivid example of the economic impact of one insect on the southern economy. Figure 2 shows the distribution of
households in the areas infested by RlFA. This figure suggests the distribution of potential losses southwide. Obviously, they are greater in counties where households are concentrated, that is, in large metropolitan areas like Dallas/Fort Worth, Houston, and Miami, for example. Florida and Texas appear to be taking the brunt of the total southwide losses (53%). Rural states like Arkansas and Mississippi tend to have their estimated losses more widely dispersed among their counties.

The nature of the losses is important. We only report on those for households. There is no reason to expect that RlFA does not affect other aspects of human life and even the total ecosystem. As an example, Salin et al. (2000) also surveyed cities, schools and golf courses. Other entities are also affected by RlFA, like businesses, airports, utilities, agriculture, and the like. Many reports have surfaced about the effects of RlFA on domesticated and wild animals, and plants (e.g., Barr & Drees 1996, Mueller et al. 1999). Because these entities are difficult to estimate, the total losses across the southern U.S. are likely to be considerably larger that what we have calculated here. Obviously, additional research is needed to elucidate losses in these other areas. Although the RlFA is disdained by most who encounter it, the ant has redeeming qualities that make it useful in the landscape (Vinson 1997), so it also has positive economic value.

Acknowledgment
Jason Kuhlman, Research Specialist for the School of Forest Resources, extracted the county household data from the Census Bureau data base.

Literature Cited

Imported Fire Ant Quarantine

Figure 1. USDA APHIS quarantined counties, 2000.
Figure 2. Density of households based on the 2000 Census. Only delineated counties were under USDA/APHIS quarantine.

Table 1. Total annual losses to households by state, for RIFA depredations, expanded from a South Carolina survey (loss estimates of $100.93 / household [95% CI ± $17.05] by Miller et al. 2000).

<table>
<thead>
<tr>
<th>State</th>
<th>Infested Counties</th>
<th>Households</th>
<th>$ Losses</th>
<th>95% Confidence Interval</th>
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MODELING SOIL TEMPERATURES USING LANDSAT 7 SATELLITE IMAGES: AN IMPORTANT VARIABLE FOR RED IMPORTED FIRE ANT RANGE EXPANSION MODELS

Matt Lane, Robert Weih, Jr., and Lynne Thompson

Arkansas Forest Resources Center, School of Forest Resources, University of Arkansas – Monticello, P.O Box 3468, Monticello, AR 71656-3468

Abstract  The focus of this study is to eventually predict soil temperatures from Digital Numbers (DN) obtained from Landsat 7 satellite imagery. The research objectives include creating two regression models; the first to predict air temperatures at a height of 10 cm above the surface, the second to predict soil temperatures at a depth of 10 and 50 cm below the surface using the predicted air temperature. All temperature data was obtained from the University of Arkansas Soil Temperature Experimental Database, and Landsat 7 images were purchased from the EROS Data Center. Older satellites have been shown to accurately predict surface temperatures as close as 1°C. Previous models predicting sub-surface temperatures from surface air temperatures have shown accurate results without needing to account for soil physical features.

Introduction

The ability to map soil and surface temperatures over large geographic areas can have numerous applications in the scientific community. Currently, cameras onboard satellites are equipped with sensors able to collect data representing the full electromagnetic spectrum. Thermal data obtained by older sensors have been previously used to show surface temperatures over the full extent of the satellite’s view. Combining this concept with common models predicting temperatures of soils from temperatures known above the soil surface would in effect indirectly give one the ability to predict sub-surface soil temperatures strictly from satellite images.

The usefulness of older satellite data is restricted because of poor image resolution. As technology has improved, so has the resolution of satellite data. The new Landsat 7 Thematic Mapper (TM) sensors have higher resolutions than previous sensors from the Landsat family, making the data more useful in analyses. Thermal data of the Landsat 7 TM have a resolution of 60m x 60m (about 1-ac).

One of the applications of this form of data can be found with the red imported fire ant (RIFA). RIFA colony growth and survival is linked to soil temperatures. With the modeling of surface temperature to soil temperature, a researcher can locate from satellite imagery areas where the RIFA would be likely to spread and survive. With RIFA preference for open areas, the 1-ac image resolution would be useful.

Our purpose for this paper is to introduce the logic and methods of the study.
Study Sites
Temperature probes have been set up in 7 locations in northwest Arkansas near Fayetteville (Figure 1) in conjunction with RIF A studies being conducted by the University of Arkansas. Each location was chosen based upon the size (ca. 300-ac), topography (mostly level), and vegetation (open fields) that are typical of the sunny habitats loved by RIF A (Figure 2). These locations allow good assessment of temperature values for the 1-ac cells of the satellite scenes.

Figure 1. Temperature probe locations

Figure 2. Probe box located in an open field
Temperature Sampling

Temperature sampling has been ongoing for approximately 2.5 years. Six of the 7 sites have 2 sets of probes spaced at least 60m apart. Each set of probes measures air temperature near the ground (10cm above the soil surface) and soil temperatures at 10 and 50cm (Figure 3). Measurements are taken hourly, stored at the site, and downloaded periodically.

![Air temperature recorder and soil temperature probe with attached recorder](image1)

Figure 3. Air temperature recorder (top), soil temperature probe with attached recorder (bottom)

Landsat 7 TM images

Landsat images cover an area of approximately 115 x 106 miles and are often referred to as scenes (Figure 4). Every 16 days the satellite passes over and records the same area at approximately 10:00 a.m. local time. 12 images have been purchased that were taken over a span of more than a year. Different seasons will be represented in these images in an attempt to get a good distribution of temperature data. The study sites do not fit in a single Landsat 7 scene, so the 12 images are composed of two different scenes that together represent all the field sample locations (Figure 5).

![Example of a Landsat 7 Scene](image2)

Figure 4. Example of a Landsat 7 Scene
Figure 5. "Footprints" of satellite images relative to study sites

Linking Sites to Pixels
All probe sites have been GPSed to find their location. Satellite scenes were ortho-rectified to the earth's surface using Imagine software and Digital Elevation Models. The digital number on the satellite scene (Figure 6) will then be associated with the correct air temperature at probe sites using Imagine software and the GPS coordinates.

Figure 6. Sample image grid showing digital numbers arranged in a gray scale from 0-255
Temperature Modeling

Two types of models are in creation. The first correlates the satellite data to the ground truthed air temperature. Extraneous variables, such as atmospheric conditions at the time of image collection, are not included in order to keep the model as simple as possible. Any data in a satellite scene that coincides geographically with clouds or heavy haze will not be included because it will not give an accurate surface temperature. Similar studies have reported high accuracies, some within 1°C of actual temperatures (Wukelic et al. 1989).

The second type of model correlates the air temperature to soil temperature at both 10cm and 50cm. Site-specific factors, like soil type, will not be included within the model. Previous studies have shown that though they can improve a model, these variables are not always necessary (Kluender et al. 1993). Both models are being developed using regression analysis. The resulting models will be tested against ground truth data withheld from model creation. A possible example of this can be found in Figure 7.

Literature Cited


Temperature at 10cm above the surface

Temperature at 10cm below the surface

Temperature at 50cm below the surface

23 °C

42 °C

Figure 7a. Example of possible temperature results when models are applied to a Landsat 7 scene (August 28, 2000)

Figure 7b. Close-up of Figure 7a draped over a Digital Orthographic Quad in SE Fayetteville, AR
BEST TIMING FOR ONE APPLICATION OF AMDRO® BAIT FOR RED IMPORTED FIRE ANT CONTROL IN THE SOUTHERN U.S.

Lynne Thompson¹, Suzanne Wiley², Michael Korzukhin³, and Sanford Porter⁴

¹ Arkansas Forest Resources Center, School of Forest Resources, University of Arkansas- Monticello, Monticello, AR, 71656-3468  
email:thompson@uamont.edu  
² University of Arkansas, Cooperative Extension Service, Arkansas Forest Resources Center, Monticello, AR 71656-3468  
³ Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya St, 35, 127276 Moscow, RUSSIA  
⁴ Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, P.O. Box 14565, Gainesville, FL 32604

Abstract  Insecticide baits are very effective in reducing populations of the red imported fire ant. Research using a computer model of red imported fire ant population dynamics (Korzukhin et al. 2001), showed that 2 to 4 applications may be needed to maintain fire ant populations at very low levels, with 1 to 3 applications needed to keep ants at moderate levels (Wiley et al. 2000). If the number of applications needed is partially answered, the next question to be asked is when is the best time to apply a bait to maximize long term control. Because our model follows the growth of each colony and its interaction with up to 500 neighboring colonies, conducting model runs to study this question is best done with a fast computer and lots of memory. Unfortunately, time and technology limitations only allowed us to model scenarios for one application per year. The results showed that either September or October is a good time if one is making only one bait application of Amdro® per year. In the deep South, November would also be fine. As we get more time, scenarios with 2, 3, and 4 applications will be conducted.

Introduction

Because of its efficacy and easy of use, Amdro® (hydramethylnon) has been a popular insecticide for red imported fire ant (RIFA, Solenopsis invicta) control for some time. Following studies in southern Mississippi, Collins et al. (1992) suggested that May/June (spring) and October/November (fall) applications provided good year-round control of fire ant colonies. Research using a computer model of red imported fire ant population dynamics, showed that 2 to 4 applications may be needed to maintain fire ant populations at very low levels, with 1 to 2 applications needed to keep ants at moderate levels (Wiley et al. 2000; Figure 1). With the question of number of applications partially answered, we then wondered when is the best time to apply a bait to maximize long-term control. To assess the best time to make a single application of Amdro, Korzukhin and Porter's (2001) model was used to conduct a more detailed assessment. That assessment, over the nearly 1.5 million km² of occupied area in the southern U.S., is reported here.

A model of fire ant population dynamics (Korzukhin and Porter 1994; Korzukhin et al. 2001) was used to reach our goal. The model describes colony area dynamics with
growth rate depending upon daily maximum and minimum soil temperatures. When the colonies are big enough and their territories touch, a specific mechanism of area interaction suppresses the growth of smaller colonies. Under natural conditions, each colony can die for several reasons, including cold weather, contacting a bigger colony, and having the queen reach her maximum age. For this study, additional mortality is added with an Amdro application.

Within the RIFA range, air temperature records at 1,863 meteorological stations were obtained from NOAA's National Climatic Data Center (NOAA 1994). Soil temperature was calculated from daily maximum and minimum air temperatures using equations developed from Chang et al. (1994) and Kluender et al. (1993). Our Amdro bait applications killed the colonies using a probability that depended on colony size. For any single Amdro application, the probability that a colony would find the bait and be killed was based on colony territory area and increased linearly from 0.8 for small colonies to 0.95 for large colonies. The model assumed that affected colonies died immediately. During a 8-yr long model run for each location, the Amdro was applied at a fixed Julian day, in the middle of a month, starting from year 2. Model output was the mean territory area for all colonies calculated over the subsequent 6 years for each Julian day (month). Model runs were made for each of the 12 months to assess the best time to make the application. Because the model follows the growth of each colony and its interaction, with up to 500 neighboring colonies, conducting model runs is best done with a fast computer and lots of memory. Unfortunately, time and technology limitations only allowed us to model scenarios for one application per year.

Output from the model for each weather station was incorporated into a Geographic Information System (GIS) using ArcView® software. Geographic coordinates for each weather station location served to link model predictions to physical locations on the maps. The most effective application date (lowest mean colony territory area calculated for each of the 12 months) was plotted for each station. Data were spatially analyzed using kriging (Burrough and McDonnell 1998) to create interpolated values for a continuous surface of application areas from the point data. Contour lines were created to delineate boundaries between application dates. A map was produced to illustrate the most effective application date necessary to maintain populations at the lowest levels. This visual representation allows easy interpretation of the complex model results.

Results and Discussion

Figure 2 shows when the lowest mean colony territory area occurred for one application over all 1,863 weather stations and calculated for each of the 12 months. It is clear that the best application time is from August to October. However, timing is likely influenced by the geographic location of the weather station. Figure 3 shows a map with contour lines created to delineate boundaries between application dates. It shows the relationship between location and timing. The results show that over most of the southern U.S. applications made between mid September and mid October provide the best control if one is making only one application of Amdro per year. In eastern Florida, applications from mid October through mid November would be fine.
Clearly, a late summer for fall application is best if only one Amdro application can be made. Notwithstanding, Fig 1 shows that one application of Amdro will not control RIFA except in the northern extent of its range or if only moderate levels of control are acceptable. So, multiple applications are needed, and, as we get more time, scenarios with 2, 3, and 4 applications will be conducted.

**Literature Cited**


Figure 1. Number of Amico applications needed to maintain RIA control at (A) 60% and (B) 90% in the southern U.S. (from Wiley et al. 2000).
Figure 2. Number of weather stations (of 1863 total) that generated the smallest colony territory area by month of treatment using one Amdro® application.

Figure 3. Best month to apply a single application of Amdro® in the southern U.S.
Abstract  The winter of 2000-2001 in Arkansas was especially cold and wet. During the subsequent spring of 2001, it was observed that red imported fire ant populations in the state had been reduced substantially from those of 2000. Although the exact cause of the population reduction is unknown, it is believed that the combination of cold and saturated soils was responsible. Theoretically, saturated soils keep fire ants above the water-line and close to the soil surface where they will be vulnerable to cold temperatures. To evaluate this thesis, we obtained max/min weather data for 54 Arkansas weather stations for the years 2000 and 2001. Weather is an important variable in our computer model (Korzukhin et al. 2001) where cold induced mortality is one of the factors influencing population dynamics. The analysis showed that our model was not very good at predicting colony mortality, but it did predict worker ant mortality reasonably well. Thus, our model probably needs to be adjusted to reflect the influence that high water tables might have on RIFA susceptibility to cold weather.

Introduction

The red imported fire ant (RIFA) (Solenopsis invicta Buren) is currently distributed throughout much of the southern U.S. where it occurs across a wide range of temperatures. In Arkansas, RIFA permanently infests much of the southern one-half of the state (pers. corr., David Blackburn, Arkansas State Plant Board). The winter of 2000-2001 in Arkansas was especially cold and wet. During the subsequent spring and summer of 2001, it was observed that RIFA populations in the state had been reduced substantially from those of 2000. Although the exact cause of the population reduction is unknown, it is believed that the combination of very cold weather and saturated soils was responsible. Theoretically, saturated soils (i.e., high water table) keep RIFA above the water and close to the soil surface where they will be vulnerable to the cold temperatures.

Recent attempts have been made to model the fire ant system to better understand the ants' dynamics (Adams 1998, Korzukhin et al. 2001). Because soil temperature is the main ecological factor determining colony metabolism and activity (Tschinkel 1993, and many others), it is used as the primary ecological factor in our model (Korzukhin et al. 2001). The purpose of this poster is to assess if our model could have predicted the
observed RIFA mortality. If the model could not, then we will plan a strategy to improve it.

Methods
We have an imported fire ant population level model (Korzukhin and Porter, 1994; Korzukhin et al., 2001) which can be applied to simulate the situation. The model assumes that the number of workers in a colony is proportional to the area of its territory. Consequently, the size of a colony is given by colony territory area. The model is driven by soil temperature only, so we would expect that the modeled mortality would be less than the observed mortality. This is because observed mortality was likely influenced by both soil temperature and soil moisture. The model includes a colony behavior function that allows the ants to move deeper into the soil during the winter. For this analysis, that function remained active.

We obtained NOAA max/min daily temperature data for 54 Arkansas weather stations for the years 2000 and 2001, and ran the model for these years with initial colony distributions obtained from runs using 1982-93 Arkansas weather data for these stations. Model output included number of colonies and the total colony territory area occupied by the colonies on a 1 ha plot. Typically, as colony size increases on the plot, total territory area controlled by all colonies increases dramatically, and correspondingly, the number of colonies able to survive decreases rapidly.

Results and Discussion
Figure 1 shows the minimum air and soil temperatures, colony territory area, and number of colonies for 2000 and 2001 at Arkadelphia where little RIFA mortality was calculated. Figure 2 shows the same information for Batesville where total RIFA kill was calculated. These two figures show the extremes. A third variation is shown in Figure 3 with plots of colony territory area and number of colonies for 3 other sites with intermediate calculated mortality. The computer experiment yielded total fire ant kill at 17 locations and moderate decreases in 23, while the remaining 14 sites showed little decrease. Checking these results against the locations of the weather stations showed the model did not eliminate colonies (or reduce them much) at many of the southern Arkansas locations where we had reports of substantial RIFA population declines. Figure 4 shows the locations of weather stations and whether the calculated mortality was low, moderate or complete. This picture shows that our model did a poor job of predicting RIFA colony mortality in southern Arkansas during the winter of 2000-2001. However, our model did predict decreases in RIFA numbers, they were just not as large as the anecdotal evidence provided. These results provide information for model improvement, namely, a temperature-precipitation interaction could be incorporated. Perhaps we can get help from new studies, like those of James et al. (2002), or additional studies may be needed to clarify the relationships between low temperatures and high water tables.

Literature Cited


Figure 1. Minimum air and soil temperatures, colony territory area, and number of colonies for 2000 and 2001 at Arkadelphia, where little RIFA mortality was calculated by the model.
Figure 2. Minimum air and soil temperatures, colony territory area, and number of colonies for 2000 and 2001 at Batesville, where complete RIFA mortality was calculated by the model.
Figure 3. Colony territory area and number of colonies for 2000 and 2001 at Benton, Jonesboro, and Stuttgart, where moderate RIFA mortality was calculated by the model. The jagged line indicates colony numbers and the smooth line colony territory area. Territory area is a better predictor of population mortality as it reflects changes in larger colonies.
Figure 4. Map of the RIFA quarantined counties in Arkansas and the weather stations showing the relative reduction in colony territory area from 2000 to 2001.
EPIZOOTIOLOGY OF A THELOHANIA SP. (MICROSPORA) INFECTING THE RED IMPORTED FIRE ANT IN MISSISSIPPI

D. A. Streett¹ and Thomas Barton Freeland, Jr.²

USDA, ARS, Biological Control of Pests Research Unit, Stoneville, MS¹
Mississippi State University, Delta Research and Extension Center, Stoneville, MS²

ABSTRACT:
Epizootiological studies were conducted of Thelohania solenopsae in natural populations of the red imported fire ant, Solenopsis invicta. There was an increase in the inter-colony prevalence of infection in fire ant populations during the course of the season. In addition, the level of infection in ants from infected mounds increased as the season progressed.

INTRODUCTION:
The first report of a microsporidian parasite in fire ants was identified as a Thelohania sp. (Allen and Buren, 1974). In 1977, the parasite was described as Thelohania solenopsae from the red imported fire ant (RIFA), Solenopsis invicta in Brazil (Knell et al., 1977). Several other Solenopsis species have been reported to be infected with a similar parasite in Argentina, Uruguay, Paraguay, and Brazil. In 1998, T. solenopsae was discovered in field-collected polygynous S. invicta colonies from Florida and was later found in polygynous S. invicta colonies in the Mississippi Gulf Coast area (Williams et al., 1998).

A pathogen survey conducted for the red imported fire ant in the Mississippi Delta established that a Thelohania sp. was present in RIFA populations located on a National Wildlife Refuge site identified as the Povall tract in Sunflower County, MS. The Thelohania sp. from S. invicta was morphologically indistinguishable from Thelohania solenopsae reported in S. invicta in Florida. The objective of this study was to investigate the dynamics of a natural infection of Thelohania sp. in RIFA populations from the Mississippi Delta.

MATERIALS AND METHODS:
After an extensive survey of the RIFA populations at the Povall tract, six RIFA mounds infected with the Thelohania sp. were identified for this study. Circular plots, each 20 meters in diameter, were established from each of the centrally-located infected mounds.

Mounds were mapped in each plot with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V2.2 software (TDS) installed on a Compaq iPAQ pocket pc. Studies were initiated in July 2000 and mounds were mapped in each plot every two months and monitored for size and presence of the Thelohania sp. Population estimates were made for each mound mapped in a plot using a population indexing system based on size and brood production. A vial sample was removed containing 100-1000 ants from each mound, labeled for identification, and stored on ice until frozen. Homogenates prepared from 20 frozen ants in a vial sample were examined visually for the presence of spores. A sample consisting of 20 ants was removed from each vial, homogenized with a pellet homogenizer in 0.25ml of distilled water and an aliquot of the suspension examined by phase microscopy.
for the presence of spores. Spore counts per microscope field were made for ten fields and then averaged. Concentrations of spores per field were assigned a score: 0=none, 1=>0-1 spore/field; 2=>1-5 spores/field; 3=>5 to 10 spores/field; 4=>10 spores/field in each sample for level of infection. Field data were entered into ArcView 3.2a Geographic Information Systems (GIS) for analysis, and for spatial and temporal presentation of the data.

RESULTS:
A total of 306 RIFA mounds was initially surveyed at the Povall tract (Fig. 1.). After establishment of the six circular plots (20 m. diameter) a total of 74 mounds remained in the study.

Preliminary results show a 2-fold increase in the overall inter-colony prevalence of infection among all of the plots (Table 1). Increases in the inter-colony prevalence of infection occurred during a subsequent collection but were less pronounced (data not shown).

Only one of the six mounds identified as infected during the first sample showed a lower score for the level of infection during the second sample. Overall, the level of infection among all of the mounds increased nearly 20% from the first to the second sample. This trend has continued for the third sample with a gradual increase in the overall level of infection for the mounds (data not shown).

DISCUSSION:
Inter-colony prevalence of infection and the overall level of infection for the RIFA mounds both show an increase by the Thelohania sp. Detailed epizootiological studies of the Thelohania sp. will lead to a better understanding of disease patterns in RIFA populations. A more thorough understanding of this disease will allow us to answer some of the more intriguing questions regarding the horizontal transmission of this parasite in RIFA populations.

ACKNOWLEDGMENTS:
The authors would like to express their appreciation to Mr. Anthony Pranschke for his assistance with this study.

REFERENCES CITED:


Table 1. Prevalence and Level of Infection by *Thelohania* sp. in Red Imported Fire Ant Populations at the National Wildlife Refuge Site

<table>
<thead>
<tr>
<th>PLOT</th>
<th>TIME 0</th>
<th>TIME 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN LEVEL INFECTION(N)</td>
<td>INTER-COLONY % PREVALENCE (N)</td>
</tr>
<tr>
<td>A</td>
<td>4.0(1)</td>
<td>3.8(20)</td>
</tr>
<tr>
<td>B</td>
<td>2.2(6)</td>
<td>40.0(15)</td>
</tr>
<tr>
<td>C</td>
<td>4.0(1)</td>
<td>50.0(2)</td>
</tr>
<tr>
<td>D</td>
<td>2.0(4)</td>
<td>50.0(8)</td>
</tr>
<tr>
<td>E</td>
<td>1.0(1)</td>
<td>11.0(9)</td>
</tr>
<tr>
<td>F</td>
<td>1.0(1)</td>
<td>7.7(13)</td>
</tr>
<tr>
<td>R</td>
<td>2.2(14)</td>
<td>21.0(67)</td>
</tr>
</tbody>
</table>
INTRODUCTION:

The red imported fire ant (RIFA), *Solenopsis invicta* has been encroaching on the range of the black imported fire ant (BIFA), *Solenopsis richteri* to the extent that the current range of BIFA is limited to only three states, northern Mississippi, northwestern Alabama, and southern Tennessee. In the state of Mississippi where these two species coexist, evidence of hybridization has been reported (Vander Meer et al., 1985). The F1 hybrids are reproductively viable and are found in a broad band across the northern tier of Mississippi, Alabama, and Georgia (Diffie et al., 1988). The objective of this study is to determine the distribution of the RIFA, BIFA, and hybrid populations in Mississippi.

MATERIALS AND METHODS:

Study Site: Samples of worker ants were collected from field colonies in northern Mississippi. Mounds were mapped with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V2.4 software (TDS) installed on a Compaq iPAQ. Population estimates were made for each mound mapped in the survey using a population indexing system based on size and brood production. A vial sample containing 100-1000 ants was also removed from each mound, labeled for identification, and stored on ice until frozen. Frozen samples of major caste workers were examined and identified to species using a key prepared by Timothy Lockley. Ant samples identified as *S. richteri* were shipped frozen to Dr. Robert Vander Meer and were analyzed by gas chromatography for venom alkaloids and cuticular hydrocarbons (Vander Meer et al., 1985). These two classes of compounds readily distinguish RIFA, BIFA and hybrid. Field data and the results from species and hybrid identification were entered into ArcView 3.2a Geographic Information Systems (GIS) for analysis, and for the spatial presentation of the data.

RESULTS:

The distribution of RIFA, BIFA and hybrid imported fire ant populations in Mississippi for 2001 are shown in Fig. 1. A total of 176 mounds were surveyed from 43 counties in Mississippi. The distribution of RIFA, BIFA and hybrid populations are shown in Fig. 1. An earlier report found hybrid populations in five counties from northeastern Mississippi (Fig. 2). In this investigation, hybrid populations were found in fifteen counties extending as far west as Bolivar county in the Mississippi Delta (Fig. 1). Earlier reports show BIFA populations in eight counties of northeast Mississippi (Fig. 2). In this investigation, BIFA populations were found in fifteen counties extending northwest to Tunica county, and as far south as Noxubee county in eastern Mississippi. In 2001, RIFA populations have been found as far north as Bolivar county in west Mississippi. Further south in Mississippi RIFA populations extend throughout the central and southern region of the state (Fig. 1). The BIFA and hybrid populations can be found further south in eastern Mississippi (Fig. 1).
Figure 1. Distribution of *S. richteri*, *S. invicta*, and hybrid populations in Mississippi for 1986, Diffie et. al. 1988.

Figure 2. Distribution of *S. richteri*, *S. invicta* and hybrid populations for Mississippi in 2001
DISCUSSION:

In our study the range of hybrid populations in Mississippi extends to fifteen counties. Although these data are preliminary, several general conclusions can be reached regarding the distribution of imported fire ants in Mississippi. RIFA populations extend further north in west Mississippi, whereas BIFA/hybrid populations extend further south in eastern Mississippi and can be found in several northwestern counties. A more extensive survey is being continued of the imported fire ant populations in Mississippi.

ACKNOWLEDGMENTS:

The authors would like to express their appreciation to Mr. Anthony Pranschke for his assistance with this study.

REFERENCES CITED:


AREA-WIDE SUPPRESSION OF BLACK IMPORTED FIRE ANTS ON PASTURE IN MISSISSIPPI

D. A. Streett¹, James T. Vogt¹, and Roberto M. Pereira²
USDA, ARS, Biological Control of Pests Research Unit, Stoneville, MS¹
USDA, ARS, Imported Fire Ant and Household Insect Research Unit, Gainesville, FL²

ABSTRACT:
A USDA-ARS area-wide demonstration project has been established against imported fire ants in pastures. Concurrent introductions of biological control agents of fire ants, e.g. microsporidia and phorid flies with registered fire ant bait products will be used to demonstrate area-wide management of fire ant populations on pastures. Biological control agents were not detected in a preliminary survey at any of the sites.

INTRODUCTION:
Imported fire ants are an important pest in the United States. This pest is widespread in the south and southwest, and has a major impact in urban, agricultural, wildlife, recreational, and industrial area's (deShazo et al., 1990; Wojcik, 1994; Lofgren, 1986). The geographic range of the imported fire ant continues to expand (MacKay and Fagerlund, 1997) and will probably continue to increase without the implementation of a centralized pro-active management program.

In 1995, the USDA-ARS implemented an area-wide pest management initiative targeted at key pests in the United States (Chandler and Faust, 1998). Recently, a USDA-ARS demonstration project for the suppression of fire ants on pastures has been established in five states: Florida, Mississippi, Oklahoma, South Carolina, and Texas. Biological control agents of fire ants, e.g. microsporidia and phorid flies will be used in conjunction with registered bait products to demonstrate area-wide management of fire ant populations on pastures. Pre- and post-treatment assessments, and environmental and economic evaluations will be made to assess the progress and success of the project.

The following study reports on the status of the USDA-ARS fire ant demonstration project in Mississippi. The impact of integrated chemical methods; Amdro® (hydramethylnon) and Extinguish ™ (Methoprene), with biological methods; a microsporidian parasite Thelohania solenopsae and a phorid fly, Pseudacteon curvatus will be assessed against the black imported fire ant (Solenopsis richteri) in pastures.

METHODS:
Four pasture sites infested with the black imported fire ant, S. richteri were selected in northern Mississippi from Grenada and Clay counties. Mounds were mapped in each plot with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V2.2 software (TDS) installed on a Compaq iPAQ pocket pc. Population estimates were made for each mound mapped in a site using a population indexing system based on size and brood production. A vial sample containing 100-1000 ants was also removed from each mound, labeled for identification, and stored on ice until frozen. In the laboratory samples were identified to species and examined for T. solenopsae spores. Four homogenate samples consisting of 5 frozen ants each were examined visually for the presence of spores using phase microscopy. The presence of Pseudacteon flies at a site was determined by inspecting a total of 20 disturbed mounds every few minutes for
hovering flies for a period of 20-30 minutes. Field data were entered into ArcView 3.2a Geographic Information Systems (GIS) for analysis, and for the spatial and temporal presentation of the data.

RESULTS:

Two sites located in Grenada county were selected for the chemical control treatment, and two sites located in Clay county were selected for the combined biological and chemical treatment. *Pseudacteon* flies were not detected at any of the sites in Mississippi. Results for the presence of *T. solenopsae* in black imported fire ants are shown in Table 1. *T. solenopsae* was not found in any of the one hundred mounds examined at each of the sites in Mississippi.

Table 1. Prevalence of infection by *T. solenopsae* in black imported fire ant populations located at four sites in north Mississippi.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SITE</th>
<th>TIME (0) INTER-COLONY % PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>Grenada County</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Torrance</td>
<td>0 (100)</td>
</tr>
<tr>
<td></td>
<td>Woodland</td>
<td>0 (100)</td>
</tr>
<tr>
<td>Chemical &amp; Biological</td>
<td>Clay County</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knox</td>
<td>0 (100)</td>
</tr>
<tr>
<td></td>
<td>Prima</td>
<td>0 (100)</td>
</tr>
</tbody>
</table>

Figure 1. Two study sites in Clay County, MS to receive chemical and biological control applications. Inner containment area is to receive chemical bait application. Outer area is to receive biological control agents *T. solenopsae* and *P. curvatus*. One hundred mounds sampled at each site for *T. solenopsae* infection, Fall 2001.
Figure 2. Two study sites in Grenada County, MS to receive chemical bait applications only. Inner containment area is to receive chemical bait application. Outer area is to receive no biological control. One hundred mounds sampled at each site for *T. solenopsae* infection, Fall 2001.

DISCUSSION:
Four sites have been identified for the USDA-ARS demonstration project in Mississippi. The biological control agents were not detected in a preliminary survey at any of the sites. Consequently, the project in Mississippi will offer a unique opportunity to study black imported fire ant populations that have not been exposed to the biological control agents.

ACKNOWLEDGMENTS:
The authors would like to express their appreciation to Mr. Anthony Pranschke for his assistance with this study.

REFERENCES CITED:


LITTER REMOVAL BY SMALL MAMMALS IN THE PRESENCE OF THREE DIFFERENT DENSITIES OF RED IMPORTED FIRE ANTS

Theresa L. Bedford¹, S. Bradleigh Vinson², and William E. Grant¹

¹Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77845-2258; ²Department of Entomology, Texas A&M University, College Station, TX 77845-2475

ABSTRACT We explored the influence of red imported fire ant (Solenopsis invicta) density on foraging activity, in the form of litter removal, by small mammals in the Post Oak savanna of Central Texas. We divided an ungrazed pasture into 3 adjacent treatment areas (an area with naturally occurring densities of S. invicta, an area with reduced density of S. invicta, and an area with increased red imported fire ant (RIFA) density). We trapped small mammals, and calculated catch per unit effort (CPUE) in each of three areas each month. We quantified RIFA density by counting the number of ants recruited to census stations in each area, and calculating CPUE. We measured amounts of litter removed from bait stations, and calculated means for each area-bait combination. Small mammals removed the most litter from the Natural RIFA Density Area, where their CPUE was highest. In most cases, litter removal by small mammals was next highest in the Low RIFA Density Area.

Red imported fire ant (S. invicta) is a successful invader for several reasons (Porter and Savignano 1990) including a wide range of climate tolerances, a broad range of food resources, rapid reproduction, and rapid colony establishment, especially in disturbed habitats (Vinson and Greenberg 1986). S. invicta is an aggressive exotic that alters the species composition of infested communities (Porter et al. 1988, Porter and Savignano 1990, Jusino-Atresino and Phillips 1994, Vinson 1994). Vertebrates may be negatively affected (Maxwell et al. 1982), as is indicated by reports of S. invicta effects on amphibians (Freed and Neitman 1988), reptiles (Landers et al. 1980, Mount et al. 1981), birds (Ridlehuber 1982, Sikes and Arnold 1986, Wilson and Silvy 1988, Pedersen et al. 1996), and small mammals (Masser and Grant 1986, Flickinger 1989, Smith et al. 1990, Killion and Grant 1993, D. K. Ferris 1994, K. P. Ferris 1994, Killion et al. 1995). In this study, we are exploring the influence of fire ant density on foraging activity, in the form of litter removal, by small mammals. Our hypothesis is that plant material and animal material litter baits accessible to small mammals will have equal removal amounts in areas with naturally occurring RIFA densities, areas with artificially high RIFA densities, and areas from which RIFA densities have been experimentally reduced.

METHODS

In an ungrazed 60 x 150 m pasture, we divided the site into 3 adjacent areas: (1) Natural RIFA Density Area — control, (2) Low RIFA Density Area — ant-toxic bait treatment of S. invicta (Drees et al., 1995), and (3) High RIFA Density Area — ant-toxic bait treatment of native ants; plus, additional colonies of fire ants. S. invicta CPUE was calculated from numbers of RIFA recruited to census stations in each area. We trapped small mammals each month for 5 consecutive nights, and small mammal CPUE was calculated for each month in each area. Litter removal stations were located in each area, and consisted of combinations of 3 different litter baits (crickets,
mealworms, or mixed seeds) and 4 different bait can designs (for ants only, mammals only, both ants and mammals, and neither ants nor mammals). Litter removal trials were run between 21 September and 21 October 1999, when the RIFA densities had reached the appropriate levels in all 3 areas. Means of litter removal were calculated for each area-bait-can combination.

**RESULTS and DISCUSSION**

This ungrazed pasture had densities of RIFA lower than a disturbed area. RIFA densities reached natural, low, and high levels in the target areas from mid-August through the end of October 1999. Mammal populations decreased in the Low and High RIFA Density Areas during late summer, possibly due to drought conditions, while the Natural RIFA Density Area remained the same. The mean litter removal by “neither mammals nor ants” is 0.15 g, which may indicate that mass is lost by all litter, due to the experimental process. In several instances, entire crickets were removed from mammal-only bait cans in the High RIFA Density Area, which may indicate foraging from these open cans by birds; excluding this, small mammals removed more litter from the Low RIFA Density Area than from the High RIFA Density Area. Small mammals removed the most litter from the Natural RIFA Density Area, where their CPUE was highest.

**References Cited**


ARE FIRE ANTS INTERFERING WITH BIOLOGICAL CONTROL OF PECAN APHIDS?

Alejandro Calixto, Allen Knutson, Marvin K. Harris, B. Ree and A. Dean.

Department of Entomology
Texas A&M University
College Station, TX 77843-2475
acalixto@tamu.edu

The impact of imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae), on pecan aphids and their natural enemies were studied in a commercial pecan orchard for two growing seasons. Two treatment and a control were established and replicated four times; 1) a *methoprene* bait treatment applied twice on 2000 and once at 2001, 2) a *chloryprifos trunk treatment applied three times on 2000 and once on 2001, and their respective untreated controls. Ants were monitored by periodic mound counts, pitfall traps and baited vials. Herbivores and their natural enemies were monitored by inspecting foliage and deployment and recovery of corrugated cardboard refuge strips. *Methoprene* bait treatment significantly reduced red imported fire ant mounds and foraging activity in up to 70% compared to the trunk treatment and control. Blackmargined pecan aphid, *Monellia caryella* (Fitch) (Homoptera: Aphidae) was the primary herbivore present during both seasons, with peak densities occurring in June, but not exceeding economic thresholds. Natural enemies, particularly lacewings, lady beetles and spiders, fluctuated in density in concert with aphids. The red imported fire ant was not shown to affect aphid densities and had little if any impact on natural enemies.
Population Decreases of the Red imported Fire Ant 
and its Relationship to Weather Conditions 
Doug Petty¹, Kelly Loftin², and Donna Shanklin³ 
University of Arkansas, Cooperative Extension Service

1. Miller County, U of A Cooperative Extension Service, 400 Laurel, Suite 215, Texarkana, AR 71854 dpetty@uaex.edu 
2. U of A Cooperative Extension Service, Environmental and Natural Resources Section, P. O. Box 391, Little Rock, AR 72203 kloftin@uaex.edu 
3. U of A Cooperative Extension Service, Environmental and Natural Resources Section, P. O. Box 3468, Monticello, AR 71656 shanklin@uaex.edu

Abstract 
Red imported fire ant (RIFA) numbers were markedly reduced in Spring 2001 in Arkansas. Analysis of the rainfall and temperature data around Texarkana, Arkansas suggests that there was over a two-week period in which the ambient temperature was at or below freezing during December 2000. A survey of Arkansas County Agents in the fall of 2001 found agents from throughout the state noted a decline in the presence of fire ants also.

Introduction 
The Red Imported Fire Ant was first discovered in Arkansas in the late 1950's. Since its introduction it has been found in over 40 counties throughout the state, of which 27 are currently in the Federal Imported Fire Ant Quarantine. The ant has spread north of the state capitol in Little Rock, to large 1000 acre tracts in Ft. Smith, east of Oklahoma. 
Temperature and moisture have cited the most often as the limiting factors involved in the spread of RIFA (Vinson, 1997). Both of these environmental factors played a role in the decline of the RIFA populations in Arkansas in 2001.

Evaluation Methods
Mound data was obtained from data collected from the Texarkana Neighborhood Fire Ant Abatement Program (Fig 1). Active mounds are counted in various 50 feet x 50 feet plots in untreated areas adjacent to neighborhoods. Counts are made prior to treatment initiation in the spring, after the first treatment in the summer, and prior to the last treatment in the fall. Weather data including rainfall and ambient temperature were obtained from data collected by the Farm Service Agency (FSA) in Texarkana, Arkansas. Figure 2 is the result of the following equation: 

\[(\frac{(ay - by)(ax - bx)}{cx - ax}) + ay = cy\] 

Where: \(a\) = starting point, \(b\) = ending point, \(c\) = unknown point, \(y\) = rain in inches, \(x\) = days.
To determine if the reduction in fire ant numbers was statewide, a survey was sent via electronic mail to the 85 county offices in Arkansas the first of September of 2001. The survey asked four questions:
1 - What would be your best estimate of the percent decline (if any) in fire ant presence in your county this year?
2 - What would you attribute the decline to?
3 - Have you noticed any increase in native ant this year?
4 - Have you noticed more fire ants this year in open areas (pastures, fields, etc.) or protected areas (adjacent to buildings, sidewalks, fences, etc)?

Discussion
Drought, saturated soils, and low temperatures all played roles in the population decreases of RIFA in 2001. The number of active mounds in the Texarkana Neighborhood Fire Ant Abatement Program were initially lower in Spring 2001, relative to the Spring 2000 (Fig 1 and 2). The Texarkana weather data (Fig 3 and 4) shows several periods of temperatures at or below freezing, with saturated soil conditions, which may have impacted RIFA.

Survey responses were from fire ant infested areas inside and outside of the Quarantine. Their responses support the Texarkana data by showing a decrease in fire ant numbers throughout the state (Fig 6). The counties responding are representative of verbal responses from other agents. Several noted the summer drought of 2000 and the cold wet weather in 2000/2001 as reasoning for the decline in fire ant numbers.

Although fire ants are omnivorous, types of food resources play a role in colony development (Porter & Tschinkel, 1987). Insufficient food resources in the summer and fall of 2000 may have resulted in decreased fire ant colony size and its' capacity to withstand additional stresses. The Arkansas RIFA decline is another example where local climate influences colony dynamics directly and indirectly, by influencing the rate of development of immature ants, and by affecting food abundance (Porter and Tschinkel 1987).

VanGelder’s (2001) evaluation of the “Korzukhin Model” (Thompson et al 1999) found that daily fluctuations in the direction of low temperature, resulted in considerable decreases in colony size, and colony alate production. Colonies going into winter have a high number of the worker caste (Tschinkel 1993).

Therefore, if large numbers of workers were killed by the freezing temperatures, they would have been unavailable to rear sufficient worker and reproductive brood to replace the dead workers.

Mounds do not provide shelter to the cold (Francke and Cokendolpher 1986), and fire ants to not have an adaptive behavior or physiology to survive extreme
cold temperatures. Phillips and Cokendolpher (1988) found the lowest point an ant would remain unfrozen was approximately 210 F.

Diffie et al. (1997) noted a decline in fire ant mound numbers in one Georgia county after a rapid decrease in temperature similar to the decrease in temperature similar to the decreases in temperature noted on December 13, 2000 in Texarkana (Fig. 4). The Arkansas RIFA decline is another example where local climate influences colony dynamics directly and indirectly, by influencing the rate of development of immature ants, and by affecting food abundance (Porter and Tschinkel 1987).

References Cited
Fig 1. Comparison of mound numbers 2000 – 2001 in Texarkana Arkansas Neighborhood Abatement Program evaluation plots.

Fig 2. Actual rainfall compared to 30 year average rainfall.

Fig 4. Comparison of accumulative rainfall (in) and high and low temperatures (F).
Fig 5. Response to Agents Survey

**Question 1**
- 50% decline
- 90% decline
- 80% until late August, above 60%
- 80% or higher
- Good decrease
- I'm not sure
- 90% or better
- Noticeable decline (80%)
- 50% decline

**Question 2**
- Hard winter & control measures
- Cold weather during winter
- Cold weather
- Due to ice last winter
- Extreme weather, conditions both drought and cold
- Hard winter
- Environment – Drought plus cold weather and treatment or baiting both these combined

**Question 3**
- Not really
- No
- Yes
- No
- Yes
- Yes
- No
- Yes

**Question 4**
- We had a serious problem at fairgrounds, and courthouse in 99-00. They were in all areas. This year they have been next to buildings and next to sidewalks
- Haven’t noticed any difference
- Adjacent to buildings and sidewalks
  - ...protected areas around the sidewalk...
- Most fire ant mounds have been noticed around buildings, sidewalks, and such things
- Near protected areas
SUMMARY OF INSECTICIDE TESTS DIRECTED AT MANAGING IMPORTED FIRE ANTS IN FIELD NURSERIES

Jason B. Oliver¹, Derek Bailey², Karen M. Vail², and Anne-Marie Callcott³

¹ Tennessee State University, Cooperative Agricultural Research Program, TSU Nursery Crop Research Station, 472 Cadillac Lane, McMinnville, TN 37110
² University of Tennessee, Department of Entomology and Plant Pathology, 2431 Center Drive, 205 Plant Science Bldg., Knoxville, TN 37996-4560
³ USDA-APHIS, Gulfport Plant Protection Stn., 3505 25th Ave., Bldg. 16, Gulfport, MS 39501

ABSTRACT

Effective treatments or management alternatives for nursery producers are needed to eliminate imported fire ants from nursery stock and satisfy quarantine requirements. Two studies pertaining to managing fire ants in field nurseries are reported.

Study A: Nursery producers in Tennessee primarily ship nursery stock during the fall - winter months when trees are dormant (October to April). Winter is the most difficult time to attempt fire ant control with insecticides, because toxicants are less likely to contact the ants due to: 1) less foraging activity, 2) greater colony depth, and 3) slower ant metabolism. Insecticides that are likely to work in the winter months are those with contact activity and the potential to move into the soil profile. Talstar granules applied in January 2001 at 1.2 and 22.4 g/m² to individual mounds gave faster control than Orthene or fipronil and was the only chemical to completely eliminate fire ants from the mound. However, three Talstar-treated mounds were recolonized between 63 and 83 days after treatment (DAT).

Study B: It is anticipated that fipronil granules may be registered in the near future for use against fire ants in ornamentals. In study B, fipronil granules applied with a broadcast spreader at 0.015 and 0.028 kg/ha in late June 2000 were evaluated against black imported fire ants in Giles County, Tennessee. The maximum percentage reduction of fire ant populations in fipronil-treated plots occurred on 365 DAT and was 89.7 ± 1.2 and 91.3 ± 8.7% (± SEM) for the low and high treatments, respectively. However, extreme spring temperature fluctuations presumably caused reduction in control plots to reach 38.2 ± 36.4% by 365 DAT. This reduction coincided with a statewide reduction in fire ant populations (Vail 2001). Consequently, fire ant reduction attributable to broadcast fipronil probably never exceeded 80%.

INTRODUCTION

Imported fire ants originally were introduced from South America around 1918 into Mobile, Alabama (Vinson 1997). The ants have rapidly expanded their range to include most of the southern U.S., California, and Puerto Rico (~129.5 million hectares) (USDA-APHIS website: http://www.aphis.usda.gov/lppq/ispmai_fireants/index.html). Unlike most of the southeast, which is infested by the red imported fire ant (Solenopsis invicta Buren), Tennessee primarily is infested with the black imported fire ant (Solenopsis richteri Forel) and the hybrid of the black and the red species. Fire ants (Solenopsis spp.) are now present in all of the southern counties of Tennessee and occupy >3.2 million hectares in the state. Fire ants are easily moved with containerized or field nursery stock, as well as hay, pine straw, or any equipment that has soil attached. Many counties in the state have already been quarantined (Haun 2000). Consequently,
any area with fire ants will be required to meet quarantine requirements before shipping nursery stock.

Fire ant research at the Tennessee State University Nursery Crop Research Station (NCRS) conducted in collaboration with The University of Tennessee Agricultural Extension Service and USDA-APHIS seeks to identify effective treatments or management alternatives for nursery producers that will eliminate imported fire ants from nursery stock and satisfy quarantine requirements.

**Study A: Fall - Winter Shipping Season: Individual Mound Treatments** - Nursery producers in Tennessee primarily ship nursery stock during the fall - winter months when trees are dormant (October to April). Winter is the most difficult time to attempt fire ant control with insecticides, because toxicants are less likely to contact the ants due to: 1) reduced foraging activity, 2) greater colony depth, and 3) slower ant metabolism. Bait products require active foraging by fire ants. Because fire ants forage optimally at temperatures ranging from 23.9 to 29.5°C (Rhoades and Davis 1967), baits typically are not effective during winter months. Insecticides that are likely to work in the winter months are those with contact activity and the potential to move into the soil profile. For quarantine treatment, any product that kills the entire colony or induces the colony to move from the nursery commodity has potential. To be adopted by nursery producers, products also must be easy to use, like granular formulations that do not require large volumes of water at application time. Several products were tested during the winter of 2000/2001 to determine their potential for quarantine treatment during the winter shipping season.

**Study B: Broadcast Fipronil** - Fipronil has been tested against red imported fire ants in numerous states south of Tennessee (Mudge et al. 2000). At rates of 0.015 and 0.028 kg/hectare, fipronil 0.0143 G typically provides > 90% control of red imported fire ants for up to 50 weeks (Mudge et al. 2000). Fipronil has not been tested against black or hybrid imported fire ants, the primary species occurring in Tennessee. It is anticipated that fipronil may be registered for use against fire ants in ornamentals in the near future.

**MATERIALS AND METHODS**

**Study A: Fall – Winter Shipping Season: Individual Mound Treatments** - Several formulations of both labeled and experimental insecticides were tested as individual mound treatments in a homeowner yard near Huntland, Tennessee. Mound location was mapped using compass direction and distance from a center pivot point. Mounds were rated active or inactive prior to treatment and at weekly intervals through 12 wk. Ant samples were taken initially from several mounds and sent to the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Gainesville, Florida to determine ant species. Samples were subsequently determined to be black imported fire ant (David Oi, pers. comm.). Insecticides were applied to individual mounds by sprinkling from a salt shaker. Treatments consisted of Orthene 75 SP (acephate) (10 ml/mound), Talstar PL G (bifenthrin) at two rates (11.2 and 22.4 g/m²), and Chipco TopChoice™ 0.0143 G (fipronil) at three rates (9.7, 14.7, and 19.6 g/m²) each applied on 31 January to 15 mounds (i.e., 15 replicates) in a randomized complete block design. Talstar and TopChoice were broadcast over a 1.2-m² area centered on the fire ant mound. Chemical treatments did not receive irrigation. Air and soil temperatures were datalogged at the site using a WatchDog Model 200 data logger (Spectrum Technologies, Inc., 23839 West
Andrew Road, Plainfield, IL 60544). Cumulative rainfall was measured between sampling dates using a bucket with a funnel in the top.

**Study B: Broadcast Fipronil** - Fipronil granules (Chipco TopChoice™ 0.0143 G) were evaluated against fire ants in an abandoned pasture located in Giles County, Tennessee. Ant samples sent to CMAVE indicated the species was black imported fire ant. Plots near 0.1-hectare size were set up with a minimum of 8 mounds per plot. Mounds in each plot were mapped from a central point. Two fipronil rates (0.015 and 0.028 kg/h) and a control treatment were replicated three times in a randomized complete block design. Chemicals were applied using an Earthway® Ev-N-Spred Model C2200APP broadcast spreader on 27 June 2000. Fire ant plots were rated initially using the Lofgren and Williams (1982) and Harlan et al. (1981) population indexing method. Plots subsequently were rated at 0.5, 1, 2, 3, 4, 9, and 12-month intervals to determine percentage population reduction. Percentage reduction for each plot was transformed using arc sine (square root [X]), and treatments analyzed for differences using analysis of variance and LSD test (α = 0.05).

**RESULTS AND DISCUSSION**

**Study A: Fall – Winter Shipping Season: Individual Mound Treatments** - Chemical treatments varied both in speed and level of ant control (Fig. 1). Talstar at both rates gave the fastest control and was the only chemical to completely eliminate fire ants from the mound. However, three mounds (10% of total) treated with Talstar had ant activity on 84 DAT, indicating ants had moved back into the mound sometime between 63 and 84 DAT. Reduction in mound activity from Orthene peaked at 87% (13 mounds inactive) on 63 DAT and remained at this level on 84 DAT. Based on our study and another study (Collins and Callcott 1995), Orthene does not appear to have residual activity against fire ants much past 30 days, probably explaining the lack of additional control.

Fipronil was the slowest acting product in the study. Mound activity gradually declined to a maximum of 73, 67, and 60% (11, 10, and 9 mounds inactive) control in the low, medium, and high fipronil rate treatments, respectively. Fipronil-treated mounds appeared to still be declining at 84 DAT based on the slope of the activity graph (Fig. 1), but mound reductions occurred in all treatments, including the control, between 28 and 63 DAT. On the 63 DAT evaluation, ants were found dead on the surface of the mound and at ~ 5 to 12 cm below the surface.

Fire ants normally move deeper into the mound as temperatures decrease. At least 13 d in March had air temperatures above 10°C, with sudden drops to around -5°C. Average air and soil temperatures reflect the fluctuating temperatures during February and March (Fig. 2). We believe the ants were trapped between the cooling upper surface and the colder soil deeper in the mound. Soil temperatures taken outside the mound environment did not drop below freezing during the study. Consequently, soil temperature outside of the fire ant mound may not be a good indicator of temperature occurring in the mound. Rainfall during this study was adequate for activation and/or movement of chemicals into the soil (Fig. 2).

**Study B: Broadcast Fipronil** - Fire ant populations in the fipronil-treated plots were significantly lower than the control plots on all sample dates, except 365 DAT (Fig. 3). The low and high rate fipronil treatments did not differ on any sample date. The maximum percent
reduction in the fipronil-treated plots occurred on 365 DAT and was 89.7 ± 1.2 and 91.3 ± 8.7% (x ± SEM) for the low and high rates, respectively. However, extreme temperature fluctuations presumably caused 38.2 ± 36.4% (x ± SEM) reduction in control plots by 365 DAT. This reduction coincided with a statewide reduction in fire ant populations (Vail 2001). Consequently, fire ant reduction attributable to fipronil probably never exceeded 80%. Fipronil was less effective at controlling black imported fire ant in this study when compared to most studies targeting fipronil against red imported fire ant (Mudge et al. 2000). If fire ants near the plot edge were avoiding fipronil exposure by foraging outside of the treated area, we would expect some form of relationship to occur between mound location and colony classification (defined as weight factor K) (Collins and Callcott 1995). No relationship was obvious. Rough terrain and odd plot size may have caused an uneven distribution of fipronil, thereby allowing foragers from some mounds to avoid exposure.

ACKNOWLEDGEMENTS
Aventis Environmental Science provided partial funding of this project and supplied the fipronil product. Valent U.S.A. Corporation provided the Orthene product. We would like to thank Ricky Alexander and Crystal Lemings (Tenn. State Univ.) and Gray Haun and Steve Powell (Tenn. Dept. Agirc.) for assisting with data collection. We would also like to thank John Ferrel (Tenn. State Univ. Agricultural Extension Service) and Kevin Rose (Univ. Tenn. Agricultural Extension Service) for their assistance with locating field sites.

REFERENCES CITED
Fig. 1. Number of active fire ant mounds among different treatments at specific sampling intervals from February to April 2001 (Franklin County, TN)
Fig. 2. Average daily temperature fluctuations (obtained from hourly datalogger readings) and total rainfall amounts between sampling dates during 2001 winter insecticide test against black imported fire ant (Franklin Co., TN).
Fig. 3. Population indices for black imported fire ant colonies treated with or without fipronil on a private farm in Giles County, TN from June 29, 2000 to June 28, 2001. Treatments with asterisks differed from other treatments on that date ($P < 0.05$; $df = 2, 4$; ANOVA for RCBD/LSD).
Evaluation of a New Hydramethylnon Bait Formulation

Kelly Loftin¹, Donna Shanklin², and Fran Tomerlin³

University of Arkansas, Cooperative Extension Service
¹Environmental and Natural Resources Section, PO Box 391 Little Rock, AR 72204
²Environmental and Natural Resources Section, PO Box 3468, Monticello, AR 71656
³523 Hwy 65 & 82, Lake Village AR 71651

Abstract

The efficacy of two new bait formulations of hydramethylnon against red imported fire ants (RIFA) Solenopsis invicta were compared to Amdro® fire ant bait. These formulations were designed for broadcast applications of 21.75 pounds per acre, yet still deliver the same amount of hydramethylnon per acre (0.0073 lbs) as Amdro® fire ant bait. Efficacy was evaluated by comparing the number of foraging RIFA collected from treated and control areas. No significant differences between the number of foraging RIFA collected from the new formulations of hydramethylnon versus Amdro® fire ant bait were found. In this study the new formulations were as effective as Amdro® against RIFA.

Introduction

Baits are currently our insecticide of choice to manage red imported fire ants (RIFA), Solenopsis invicta (Hymenoptera: Formicidae) (Taber 2000) and have been used since the 1960’s (Lofgren et.al. 1975). Many effective bait products are broadcast at low rates of 1 to 1.5 pounds per acre and are very effective when correctly applied at these rates. Although methodology to apply low rates is readily available, some consumers have difficulty in achieving a uniform broadcast of 1 to 1.5 pounds per acre. These consumer tend to either apply more than the labeled rate or make spotty applications. Two experimental formulation of hydramethylnon were evaluated to address the problem. These formulations were applied at 21.75 pounds per acre yet still deliver the same amount of hydramethylnon as the standard Amdro® formulation (0.0073 lbs/acre).

Materials and Methods

Experimental Bait Formulations: Amdro A contained 0.0335% hydramethylnon formulated on corn grits plus soybean oil. Amdro B was a blend of 1 part Amdro Fire Ant Bait (0.73% hydramethylnon) and 20 parts corn grit (no additional soybean oil) to achieve a 0.0335% hydramethylnon formulation. Amdro FAB was simply retail Amdro® Fire Ant Bait. All formulations of hydramethylnon bait were supplied by AMBRANDS.
**Insecticide Applications**: Prior to treatment RIFAs were actively foraging as determined by use of bait stations. Bait formulation and application rates were as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent ai</th>
<th>Lbs ai/acre</th>
<th>Lbs/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Control</td>
<td>0.0335%</td>
<td>0.0073</td>
<td>21.75</td>
</tr>
<tr>
<td>2 - Amdro A</td>
<td>0.0335%</td>
<td>0.0146</td>
<td>43.50</td>
</tr>
<tr>
<td>3 - Amdro A</td>
<td>0.0335%</td>
<td>0.0073</td>
<td>21.75</td>
</tr>
<tr>
<td>4 - Amdro B</td>
<td>0.0335%</td>
<td>0.0073</td>
<td>21.75</td>
</tr>
<tr>
<td>5 - Amdro FAB</td>
<td>0.73%</td>
<td>0.0073</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Baits were broadcast using a Herd seeder mounted on a Kawasaki mule equipped with a digital speedometer (Fig 1). After calibration the specified rates were broadcast by matching to the appropriate ground speed.

**Evaluation**: Pre and post treatment evaluations were conducted using bait stations to collect foraging ants within each plot. Bait stations consisted of a 1/4 inch “hot dog” cube placed on a snap vial lid and marked with a wire survey flag. Ten bait stations in each plot were made available to foraging ants for approximately 30 minutes. Estimates of the number of RIFA were recorded from each station and used to evaluate product efficacy (Fig 2).

**Statistical Analysis**: Data collected from bait stations were analyzed using Analysis of Variance based on a RCB design with 4 replicates. The protected LSD procedure was used to determine significant differences in the mean number of foraging RIFAs collected from the various treatments (Statistix 7 2000).
Results and Discussion

The mean number of foraging RIFAs collected from pre-treatment through 63 day post-treatment are summarized in Table 1. At one day post-treatment significantly fewer RIFAs were collected from the all hydramethlynon treated areas when compared to the control. These results were somewhat unexpected. We routinely detect reductions in foraging ants at 5 to 7 days post-treatment but normally a reduction is not noted at 1 day post-treatment. Results from 3 through 63 day post-treatment demonstrated the same trend except that the reductions were greater. No significant differences in control between differing hydramethylnon formulations or rates were noted for any of the evaluation periods. The percent reduction in foraging RIFA remained high (90-99%) throughout most of the study (Fig 3).

Table 1. Mean* number of RIFA collected from hot dog baited traps.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>52.8A</td>
<td>73.0A</td>
<td>73.3A</td>
<td>72.5A</td>
<td>70.8A</td>
<td>35.9A</td>
<td>71.2A</td>
<td>38.1A</td>
</tr>
<tr>
<td>Amdro A</td>
<td>68.5A</td>
<td>15.5A</td>
<td>2.3A</td>
<td>0.2A</td>
<td>0.3B</td>
<td>1.7B</td>
<td>1.4B</td>
<td>7.3B</td>
</tr>
<tr>
<td>Amdro Ax2</td>
<td>55.1A</td>
<td>1.0B</td>
<td>0.3B</td>
<td>0.0B</td>
<td>0.1B</td>
<td>0.8B</td>
<td>0.8B</td>
<td>3.7B</td>
</tr>
<tr>
<td>Amdro B</td>
<td>71.2A</td>
<td>7.5B</td>
<td>0.3B</td>
<td>0.8B</td>
<td>0.2B</td>
<td>3.2B</td>
<td>0.8B</td>
<td>3.6B</td>
</tr>
<tr>
<td>Amdro FAB</td>
<td>61.5A</td>
<td>4.2B</td>
<td>2.0B</td>
<td>0.0B</td>
<td>0.0B</td>
<td>1.3B</td>
<td>0.0B</td>
<td>4.7B</td>
</tr>
</tbody>
</table>

* Means followed by the same letter within a column are not significantly different ($\alpha = 0.05$; protected LSD)

![Fig. 3. Percent reduction of foraging RIFA in various hydramethylnon treatments.](image-url)
Results suggest that both new formulations of hydramethylnon are as effective as Amdro® against RIFA. With further study new formulations could be a viable option for consumers that have difficulty in applying fire ant baits at the 1 to 1.5 pound rate.

References Cited

Taber, S. W. 2000. Fire Ants, Texas A & M University Press, pp. 141-158
CHARACTERIZATION OF A PUTATIVE TENDING PHEROMONE IN 
SOLENOPSIS INVICTA

Colin Brent & Ed Vargo

Department of Entomology, North Carolina State University

Abstract
As with many social insects, colony function in the imported fire ant, Solenopsis invicta, is regulated by the release of chemosignals produced by mature queens. One important pheromone, released by the poison and postpharyngeal glands, promotes tending behavior by nestmate workers, enhancing the frequency of interactions such as antennal palpation, grooming and feeding. Both alpha pyrone and invictolide have been previously implicated as potential components of such an attractant, but tests of synthetic versions of these compounds indicate that individually they have little affect on worker behavior. This study identifies additional active components of this queen recognition pheromone that are necessary to elicit the tending behaviors. Pheromonal extracts made from homogenized mature queens were purified and fractionated using HPLC to isolate individual components. Component blends were tested for their relative attractiveness using a modified version of the “test arena” bioassay to determine which chemical components of the extracts were active. GC-MS was used in chemical identification of these active components. It was found that the attractant pheromone consists of 3 active HPLC fractions. The first, occurring at 3-4 minutes is composed of several alkanes and alkenes. The most relevant appear z-9-pentacosene and z-9-tricosene. A fraction at 8-9 minutes has been identified as 1-pentenyl alpha pyrone. The final fraction at 11-12 min is believed to be invictolide. Individually, alpha pyrone and invictolide don’t significantly influence behavior, but when either one or both are presented together with the alkenes, there is a very strong activational effect. Synthetic blends of these components are now being tested.
RANGE OF THE HYBRID IMPORTED FIRE ANT IN GEORGIA

Stan Diffie¹, Robert K. Vander Meer², and Wayne Gardner¹

¹ The University of Georgia Department of Entomology
² USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology

INTRODUCTION- Hybridization between the red imported fire ant, Solenopsis invicta Buren, and the black imported fire ant, S. richteri Forel, was first detected in east central Mississippi (Vander Meer et al. 1985). Although S. richteri was introduced into the United States before S. invicta, its range was restricted to only a few counties in northeastern Mississippi and northwestern Alabama. Solenopsis invicta infested most of the Southeast by that date. The hybrid forms that were first collected were from those areas where the ranges of the two introduced species met.

Diffie et al. (1988) subsequently reported the hybrid from 10 counties in western Georgia and 22 counties in north central Alabama in surveys conducted in 1985-6. The majority of those survey sites were removed from the S. richteri range. In fact, the first reported occurrence of the hybrid in Georgia (Floyd Co., 1985) was not adjacent to the range of either S. richteri or S. invicta.

Vander Meer (1986) reports that the venom alkaloid and cuticular hydrocarbon analyses readily distinguish S. richteri from S. invicta and that the hybrid is a distinct mixture of the two parent species. Ross et al. (1987) demonstrated several biochemical and genetic differences and similarities among S. richteri, S. invicta, and the hybrid. They noted that the hybrid was reproductively viable and that it had probably existed in its range for several years.

The imported fire ant now occupies all 159 of Georgia’s counties. The purpose of the study reported here was to determine the occurrence of the hybrid within this distribution within Georgia.

PROCEDURES- A grid of 149 27 km X 27 km squares was superimposed to scale on a map of north Georgia. The grid extended west to the Alabama border, north to the Tennessee border, east to a line from Wrightsville to near Elberton, and southward to a line below Macon.

If possible worker ants were collected from 3 randomly selected colonies at or near the intersect on the grid. The colonies were approximately 1.6 km apart at each intersect. These sites were surveyed during 1999-2000.

Approximately 30 workers were collected from each colony sampled by inserting a 16 X 100 mm disposable culture tube into the mound and capping it after at least 30 workers entered the tube. Collected workers were returned to the laboratory and placed in hexane for 24 – 48 hours after which they were transferred to ethanol for preservation. The hexane was evaporated, and the resulting residues were transported to CMAVE in...
Gainesville, FL. The samples were reconstituted and analyzed for venom alkaloids and cuticular hydrocarbons using gas chromatography as described in Diffie et al. (1988).

RESULTS AND DISCUSSION- Workers were collected from 435 colonies from 79 counties during this 2-year survey. Of these colonies, 188 (43%) were found to be hybrid ants. In general, the hybrid occurs west and north of a line marked by Interstate 85 as it crosses the state from near Anderson, SC, to LaGrange, GA (Fig. 1).

![Fire Ant Distribution Map](image)

Fig. 1. Distribution of hybrid fire ants in Georgia 1999-2000.

The hybrid was collected from several sites outside of that generalized range. These were in Butts, Lamar, and Washington Counties. Likewise, *S. invicta* was collected from Polk and Haralson Counties, an area totally surrounded by the hybrid. These isolated occurrences were likely due to human intervention.

The rapid spread of the imported fire ant throughout Georgia apparently resulted from the northward spread of the red imported fire ant, *S. invicta*, coupled with an eastward spread
of the hybrid form. The current distribution of the hybrid form and *S. invicta* in the northern part of the state (Fig. 1) shows a distribution of these ants that would explain such an occurrence.

This can be further corroborated by comparing this distribution data to 2 previous surveys for the hybrid in the northwestern parts of the state. In the 1985-6 survey reporting the original occurrence of the hybrids in Georgia, Diffie et al. (1988) collected hybrids from 10 counties (Fig. 2), most of which bordered a 22-county area in Alabama infested with the hybrid. Hybrids were collected from 17 additional sites in 10 counties east of that original range in 1993-4 (Fig. 3). This depicts a natural spread for the form from Alabama through northwestern Georgia.

![Fire Ant Distribution](image)

Fig. 2. Distribution of hybrid fire ants in Georgia 1985-6.
Fig. 3 Hybrid fire ant collection sites in Georgia 1993-4.

REFERENCES CITED-


2002 IMPORTED FIRE ANT CONFERENCE REGISTRANTS

ALLEN, CRAIG R
SC COOPERATIVE RES UNIT
G-27 LEHOTSKY HALL
CLEMSON SC 29631
(864) 656-4461

ALLEN, DOUGLAS R
FIRE ANT MANAGEMENT
1200 BRIAR LAKES RD
WATKINSVILLE GA 30677
(706) 353-7577
FIREANT@BELL1.SOUTH.NET

AMES, LISA
UGA
1109 EXPERIMENT STREET
EAST GRIFFIN GA 30223
(770) 223-6114

ANDALORO, JOHN T
DUPONT
45 STATEN DRIVE
HOCKESEE DE 19707
(302) 451-4743
JOINTANDALORO-1@US.A.BUPONT.COM

ANDERSON, ALVIN
BAYER CORPORATION PMB 357
1029 PEACHTREE PKWY
PEACHTREE CITY GA 30269
(770) 632-9104
MIP.ANDERSON@BAYBR.COM

AVISON, JEFFREY
AMBRANDS
2255 CUMBERLAND PARKWAY
ATLANTA GA 30339
(770) 333-8999
JAVISON@AMBRANDSPRODUCTS.COM

AZHAR, MOHAMMAD
CA FOOD & AGRICULTURE
9482 CENTRAL AVE
GARDEN GROVE CA 92844
(714) 708-1910
MAZHAR@CIFA.CA.GOV

BAILEY, DEREK W
UNIV OF TENNESSEE
5800 CENTRAL AVE PK
KNOXVILLE TN 37912
(865) 974-5173
DRBAILEY@UTK.EDU

BAKER, KARL
COACHELLA VALLEY MOSQUITO CNTR
43420 TRADER PL
INDIO CA 92201
(760) 342-1960
BAKERKARL@AOL.COM

BALL, WILLIAM L
HILTON HEAD LABORATORIES
RR 1 BOX 707
RIDGELAND SC 29936
(843) 987-3100
WHLABS@HARGRAY.COM

BARR, CHARLES L
TEXAS COOPERATIVE EXTENSION
PO BOX 2150
BRYAN TX 77806
(979) 845-6800
C-BARR@TAMU.EDU

BECKLEY, PATRICIA
LSU
202 KNAPP HALL
BATON ROUGE LA 70894
(225) 578-2180
PBECKLEY@AGCTR.LSU.EDU

BEDFORD, THERESA
TEXAS A&M UNIVERSITY
1811 C ARNOLD RD
COLLEGE STATION TX 77845
(979) 845-5702
TBEDFORD@TAMU.EDU

BERTAGNOLLI, VICKY E
AUBURN UNIVERSITY
301 FUCHESS HALL
AUBURN AL 36849
(334) 844-2530
BERTAV@ACESAG.AUBURN.EDU

BHATKER, AWINASH P
TEXAS DEPT OF AG
2913 RAYADO COURT
COLLEGE STATION TX 77845
(512) 463-5025

BLACKBURN, DAVID J
ARKANSAS STATE PLANT BOARD
1 NATURAL RESOURCES DR
LITTLE FLOCK AR 72756
(501) 225-1598
DAVID.BLACKBURN@AAR.STATE.AR.US

BORGOGNI, RICK
DOW AGROSCIENCES LLC
PO BOX 578
TALBOTT TN 37877
(423) 736-6020

BOWEN, RICHARD A
ORANGE CO FIRE ANT AUTHORITY
20541 PASCAL WAY
LAKE FOREST CA 92630
(949) 452-9242
RBOWEN@OCFIREANT.COM

BRENT, COLIN S
NCSU
736 SMALLWOOD DR #A-4
RALEIGH NC 27605
(919) 515-3784
CSBRENT@HOTMAIL.COM

BRIANO, JUAN
US EMBASSY
C/O AGRIC COUNSELOR UNIT 4325
APO AA 34034
JABRIANO@MAIL.RETINA.ALI

BRINKMAN, MARK A
UNIVERSITY OF GEORGIA
1109 EXPERIMENT ST
GRIFFIN GA 30223
(770) 412-4043

BROOKS, GARY
AVENTIS
207 SHERRY TRAIL
WEATHERFORD TX 76086
(817) 594-7164

BROWN, CHARLES E
600 SOUTH 7TH STREET
SUITE 4
OPELIKA AL 36801
(334) 749-3353

BROWN, NATHAN
MS DEPT OF AG & COMMERCE
1274 JUPITER RD
BRAXTON MS 39044
(601) 847-0353
NATHAN@MSDC.COM

BURT, STEPHEN
TECH PAC LLC
21 WINTERGREEN CT
WARWICK NY 10990
(201) 307-3374
STEVR.BURT@AVENTIS.COM

CALIXTO, ALEJANDRO
DEPT OF ENTOMOLOGY
TEXAS A&M UNIVERSITY
COLLEGE STATION TX 77843
(979) 845-3412
ACALIXTWTAMU.EDU

CALLCOTT, ANNE-MAW A
US DEPT OF AGRICULTURE
3505 25TH AVENUE
BLDG #16
GULFPORT MS 39501
(228) 822-3100
ANNE-MARIE.A.CALLCOTT
USDA.EDU
<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Phone Number</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>RENTHAL, ROBERT D</td>
<td>UNIV OF TEXAS 6900 N LOOP 1604 W BIOLOGY DEPT SAN ANTONIO TX 78249 (210) 458-5452</td>
<td>(210) 458-5452</td>
<td><a href="mailto:RRENTHAL@UTSA.EDU">RRENTHAL@UTSA.EDU</a></td>
</tr>
<tr>
<td>RICE, STANLEY A</td>
<td>SOUTHEASTERN OSU STATION A DURANT OK 74701 (580) 745-2688</td>
<td></td>
<td><a href="mailto:SRICE@OSU.EDU">SRICE@OSU.EDU</a></td>
</tr>
<tr>
<td>RIGGS, NATHAN L</td>
<td>TEXAS COOPERATIVE EXTENSION 3355 CHERRY RIDGE #212 SAN ANTONIO TX 78230 (210) 467-6575</td>
<td></td>
<td><a href="mailto:N-RIGGS@TAMU.EDU">N-RIGGS@TAMU.EDU</a></td>
</tr>
<tr>
<td>ROBERTSON, MALCOM J</td>
<td>CLEMSONS UNIVERSITY 511 WESTHOUSE ROAD PENDLETON SC 29670 (864) 646-2134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIGGS, NATHAN L</td>
<td>TEXAS COOPERATIVE EXTENSION 3355 CHERRY RIDGE #212 SAN ANTONIO TX 78230 (210) 467-6575</td>
<td></td>
<td><a href="mailto:N-RIGGS@TAMU.EDU">N-RIGGS@TAMU.EDU</a></td>
</tr>
<tr>
<td>SANDERSON, SHERRA A</td>
<td>NM DOA PO BOX 30005 MSC 38A LAS CRUCES NM 88003 (505) 646-3207</td>
<td></td>
<td><a href="mailto:SAMSANDER@NMSU.BUBBA.NMSU.EDU">SAMSANDER@NMSU.BUBBA.NMSU.EDU</a></td>
</tr>
<tr>
<td>SCHEUTZ, CLAY W</td>
<td>SYNGENTA CROP PROTECTION 7145 58TH AVE VERO BEACH FL 32967 (561) 567-5218 123</td>
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<tr>
<td>SCHEUTZ, CLAY W</td>
<td>SYNGENTA CROP PROTECTION 7145 58TH AVE VERO BEACH FL 32967 (561) 567-5218 123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCHROEDER, MICHAEL D</td>
<td>TEXAS TECH UNIVERSITY BOX 42122 DEPT OF PLANT &amp; SOIL SCIENCE LUBBOCK TX 79409 (806) 742-2828</td>
<td></td>
<td><a href="mailto:MDSCHROEDER@YAHOO.COM">MDSCHROEDER@YAHOO.COM</a></td>
</tr>
<tr>
<td>SCHROEDER, KERINNE M</td>
<td>TEXAS A&amp;M UNIVERSITY 2124 BLOCKER AG ECONOMICS COLLEGE STATION TX 77843 (979) 862-4828</td>
<td></td>
<td><a href="mailto:KMSCHROEDER@TAMU.EDU">KMSCHROEDER@TAMU.EDU</a></td>
</tr>
<tr>
<td>SHANGLIN, DONNA R</td>
<td>UNIVERSITY OF ARKANSAS PO BOX 3468 MONTICELLO AR 71655 (870) 460-1893</td>
<td></td>
<td><a href="mailto:SHANGLIN@UMONTO.EDU">SHANGLIN@UMONTO.EDU</a></td>
</tr>
<tr>
<td>SHERMAN, JOHN</td>
<td>ADE PEST CONTROL INC PO BOX 919 WINDER GA 30680 (770) 724-4454</td>
<td></td>
<td><a href="mailto:ASHER@ADEPESTCONTROL.COM">ASHER@ADEPESTCONTROL.COM</a></td>
</tr>
<tr>
<td>SIMMONS, SALLY F</td>
<td>BAYER ADVANCED LLC 5690 58TH AVE VERO BEACH FL 32967 (561) 562-6549 236</td>
<td></td>
<td><a href="mailto:SALLY.SIMMONS.B@BAYER.COM">SALLY.SIMMONS.B@BAYER.COM</a></td>
</tr>
<tr>
<td>SIMS, STEVEN R</td>
<td>WHITMIRE MICRO-GEN 3568 TREE CT INDUSTRIAL BLVD SAINT LOUIS MO 63122 (636) 861-4264</td>
<td></td>
<td><a href="mailto:STEVE.SIMSAJ@W.COM">STEVE.SIMSAJ@W.COM</a></td>
</tr>
<tr>
<td>SLATT, JOAN</td>
<td>USDA-ARS 5601 SUNNYSIDE AVENUE BLDG 4 ROOM 2172 BELTSCVILLE MD 20705 (301) 504-4700</td>
<td></td>
<td><a href="mailto:RMR@ARS.USDA.GOV">RMR@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>SMITH, WAYNE A</td>
<td>OKLAHOMA STATE UNIVERSITY BRYAN COUNTY EXT OFFICE BOX 749 COMMUNITY BLD DURANT OK 74702 (580) 924-5312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOKOLOVA, JULIA</td>
<td>LOUISIANA STATE UNIVERSITY 402 LIFE SCIENCES BLD ENTOMOLOGY DEPT BATON ROUGE LA 70803 (225) 578-1849</td>
<td></td>
<td><a href="mailto:JUNIP@CRO.COM">JUNIP@CRO.COM</a></td>
</tr>
<tr>
<td>SPARKS, BEVERLY L</td>
<td>UNIVERSITY OF GEORGIA ATHENS GA 30602 (706) 542-3179</td>
<td></td>
<td><a href="mailto:ESPIRKS@ARCHES.UGA.EDU">ESPIRKS@ARCHES.UGA.EDU</a></td>
</tr>
<tr>
<td>STAFFORD, CHRISTOPHER J</td>
<td>CROSS FALLS HOUSE PINE TREE DR W QUEENSLAND 4563 AUSTRALIA <a href="mailto:CHRISSIM@EMAIL.COM">CHRISSIM@EMAIL.COM</a></td>
<td></td>
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